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SERO-DIAGNOSTIC METHODS FOR PHYTOPATHOLOGICAL STUDIES

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Introduction

In medical pathology application of serological methods has been a great boon to pathologists for the rapid diagnosis of the syndrome of many diseases of doubtful etiology; and other diseases like syphilis, by the Wasserman's test, diagnosis of typhoid fever by Widal test are also too well known. Attempts for an early diagnosis of the menace of virus infection in animals, such as the Rabies of dogs, Foot and mouth-disease of cattle, Rinderpest of livestock, Ranikhet disease of fowls through serological methods are in vogue. Infectious virus diseases of plants such as the tobacco mosaic virus, potato virus 'X', to mention only a few, are being diagnosed serologically. Many of the conditions known as " breaking in tulips " were diagnosed as viruses by serological methods.

Plants unlike animals do not recover from virus infections and display immunity subsequently. In fact they have no circulatory system and there is lack of evidence of their possessing anything equivalent to the antibody-forming mechanism of animals. Most diseased plants, particularly those infected by viruses, do not recover but remain infected until they die although apparent recovery termed " acquired immunity " is known in tobacco ring spot virus and here the symptoms are not noticeable in the young shoot but the tissues show a virus content.

The young and growing field of plant virus serology has shown considerable vigour in recent years characteristic of any discipline which has a wide application in many agricultural areas where plant viruses take a severe toll of crop plants (Bawden, 1950; Smith, 1951; Matthews, 1957). The present attempt is to acquaint the plant pathologist in India the numerous uses of serology, particularly at a time its introduction in India, is being contemplated.

Apart from the application of serology in diagnostic medicine in India, this elegant method has hardly been used in the diagnosis and classification of plant viruses except in a few instances (Desai, 1935;

Patel, Dhande and Kulkarni, 1951). This short account is, therefore, presented for the benefit of students of this discipline and is expected to aid them in understanding the subject of plant viruses in a fuller perspective than merely understanding symptomatology, method of transmission, etc.

Methods in Serology

Serology is the branch of science dealing with immunology pertaining to the preparations, use and reactions of serums (the watery portions of animal fluid remaining after blood coagulation). Thus two essentials in a serological reaction are:

(i) An *antibody* is any substance which makes its appearance in the blood serum or body fluids of an animal, in response to the stimulus provided by the parenteral introduction of an antigen into the tissues. The precise mechanism by which antibody formation takes place is unknown, although it is generally believed that antigen accumulates in the cells of the reticulo-endothelial system of animal. Antibody is known to occur in higher concentration such as the spleen, liver, bone-marrow, in which reticulo-endothelial tissue is concentrated. The chief producers of antibody are the immature plasma cells in reticulum system. The present view is that sites of antibody production depend on the animal species and on the nature of the antigen as well as the route by which it is administered. The intensity of production at any site may bear little relation to the concentration of reticulo-endothelial tissue there (Oakley, 1959). Some workers have increased the antibody production by using substances called *adjuvants*. Mineral oil, peanut oil (groundnut oil), sesame oil, liquid paraffin (Anderson, 1955) or similar substances and non-pathogenic bacteria, which excite antibody formation, are the most commonly used as adjuvants.

(ii) An *antigen* is any substance which when introduced in unaltered condition into the animal tissue stimulates the production of antibody and which, when mixed with that antibody, reacts specifically with it in some observable way. It must bear two essential attributes, namely, power of stimulating the antibody production and the specificity of reaction with the antibody. Antigens are normally large molecules in the nature of proteins and polysaccharides. The first indications of antigenicity of plant viruses was discovered by Dvorak (1927) and specific proof of such antigenicity of plant viruses was provided by Purdy Beale (1928, 1929, 1931) with tobacco mosaic virus. Later fundamental works of Chester (1935) in the U.S.A., Bawden (1948), Kleczkowski (1941), and Smith (1951) in the United Kingdom and van Slogteren, E. (1945) in Holland helped greatly in establishing critical serological methods in identifying individual viruses and also detecting group reactions and relationships among plant viruses both in the laboratory and in the field.

That some plant viruses are good antigens, can be considered on the fact that all plant viruses are nucleoproteins with ribonucleic acid

(Stanley, 1936; Bawden and Pirie, 1936; 1937 *a*). This is also suggestive of uniformity in their fundamental composition. However, the present concept regarding the definite arrangements of these, particularly in the rod-shaped tobacco mosaic virus, is that there is a central core of nucleic acid corresponding to the lead of a pencil, with a protective outer covering of protein shell corresponding to the wood of a pencil. Further, that the former is essential for infection, while the latter is concerned with the serological properties, presents a new situation as to the serological behaviour of virus (antigen).

Preparation of Antisera

The detailed procedures for the preparation of antisera are given by Bawden (1950) besides, illustrated accounts of the techniques by Smith (1951) and Matthews (1957). Here, only the salient features of the methods will be dealt with.

The infective sap is clarified either by heating or low-speed centrifugation process or by a combination of the two methods. In the former process, the infective sap is heated to 55° C. or below for 10 min. followed by immediate cooling. Centrifugation of this treated sap yields a clear infective supernatant, which may be further dialysed to remove any more of the impurities. In the latter method, the frozen infected leaves are thawed, crushed and squeezed to obtain infective juice. This juice is centrifuged at low speed to remove the plant materials and obtain the clear brown supernatant infective sap.

The purification of the sap is done by a combination of repeated precipitation of infective sap with ammonium sulphate (a process called salting out) and low-speed centrifugation of this sap after precipitation. These processes yield a good infective juice which when injected into the animal is harmless but produces the antibodies. The sap clarification can also be effected by addition of sodium phosphate and centrifugation.

A rabbit, weighing about 2 kg. preferably with long ears having prominent veins to facilitate injection, is selected for immunization. After clearing the area of the ear veins and sterilising it with alcohol, a thin needle (size No. 14) of a hypodermic syringe of 5–10 ml. capacity (preferably of all-glass type), containing 2–3 ml. of the clarified virus (1–10 mg. of purified virus preparation) per injection, is inserted into the vein close to the lower tip of the upper surface of the ear, and the liquid (1–2 ml.) is slowly delivered. On removing the syringe, bleeding, if any, is stopped by applying saline, or holding the ear with pressure. Successive injections are given close to the earlier ones and in all, a course of 6–8 injections at definite intervals are given to the animal, more often intravenously than intraperitoneally. Following the last injection, after a period of ten days' rest, the animal is bled near the ear. It is not necessary to obtain the sterile sera for experimental work with plant viruses by heart puncture technique. The clear serum is obtained after centrifuging the blood for 10 min. at 2,000–3,000 r.p.m. or by

allowing the blood to clot. The sera are preserved in 0·1% phenol or 50% glycerol and kept in a cool place.

Serum is normally a watery fluid. This serum containing the antibodies is referred to as the antiserum, which can be used to detect and identify antigens. A serum is called *normal* when the animal from which it is obtained has never been previously injected with any antigenic material. *Homologous* antisera are formed by the stimulation of particular antigens used when such antisera obtained will react specifically with those antigens. *Heterologous* antisera are obtained in response to stimulation with other antigens and give a positive reaction only when the viruses used for production of different antisera have common antigen. *Polyvalent* antisera developed in Holland are used to test in one reaction the presence of several potato viruses, *X*, Y, S, and aucuba mosaic virus.

Viruses are more highly antigenic than normal plant constituents which also act as antigens. When antisera are prepared against clarified sap or in partially purified virus preparation, they should be tested against healthy extracts to determine whether they also react with them and if they do, the antibodies to normal plant constituents should be removed. This is done by absorbing the antisera with healthy plant sap. The serum is mixed with 2–5 times its volume of sap and allowed to stand overnight and the precipitate formed is removed by centrifugation.

Besides the use of rabbits, antisera against plant viruses have been produced in guinea-pigs (Chester, 1936), fowls (Newton and Edwards, 1936) and in horses (van Slogteren, E., 1955). The specific act of combination involving a reaction between the antiserum and the antigen in an observable way (normally with turbidity due to precipitate), forms the basis of serological tests, which could be demonstrated in several ways:

(1) Precipitation reaction or Precipitin test or the Tube test,

(2) Complement fixation,

(3) Anaphylaxis, and

(4) Neutralization of infectivity.

The last two methods are of little general use for work with plant viruses. The types of serological tests are considered individually hereunder.

Types of Serological Methods

(1) *The Precipitation Reaction*

The simplest test depends on the formation of a visible precipitate due to the combination of a soluble antigen and its antibody when the two are mixed in suitable proportion in the presence of an electrolyte (saline) and warmed in a water-bath at 37–50° C. A more detailed account of this reaction is mentioned here, which is more often used as a serological method for dealing with plant viruses. The precipitation

reaction is carried out in thick-walled short test-tubes 7 mm. in diameter adding 1 ml. of diluted antiserum to 1 ml. of antigen (virus solution), all dilutions being made in 0·85% saline. The antigen and antibody are mixed quickly and the tubes are placed in a water-bath at 37–50° C. with their fluid columns half immersed in it, allowing the continuous stirring of the contents by convection currents, thus accelerating the precipitation. The water-bath is illuminated from behind by a strip of light with glass front to facilitate easy observation of a visible precipitate forming in tubes within minutes after placing the tubes. The usual practice to express the results of precipitin tests with serial dilutions of reagents is by a series of *plus* (+) signs, indicating the approximate relative amount of the specific precipitate formed. There is a limiting dilution for any antiserum or any virus preparation beyond which a visible precipitate does not occur with the virus, this limiting dilution is known as *virus end-point*; for the antiserum, as the *titre* of the serum.

The precipitation reaction is of great use in quantitative studies for keeping the antiserum constant, through a progressive serial dilution by which a good estimation of virus present can be made. Noting the time of precipitation, it would be easy to obtain the precipitation end-points of the antigen, which will be the highest dilution of antigen showing the precipitate. This can be expressed as the relative concentration of the virus in the tissues. Conversely, keeping the antigen constant, through a progressive serial dilution of antiserum, it is possible to obtain the titre value of the antiserum which would be the highest dilution of the antiserum, where a precipitate is obtained at a known time after incubating in a water-bath.

Some precautions are necessary in using precipitin tests. However carefully the crude sap is clarified, spontaneous non-specific precipitation of plant constituents (mainly normal plant proteins) is liable to occur which would obscure the precipitin test. The likelihood of this occurring depends on the age, metabolic condition and species of plant and the method used for clarification. Increased temperature increases the chances of non-specific precipitation. Heat treatment of saps at 55° C. for 5 min. usually removes unstable components in the form of bulky green coagulum which is readily removed by centrifuging and a clear, stable supernatant can be obtained. If, however, it is not possible to use heat clarification because the virus is unstable, a temperature near 37° C. should be used for incubation.

It is not possible generally to distinguish between the spontaneous and specific precipitation since the former is loose and rapidly formed, as opposed to well-formed, definite precipitate in the latter. In doing critical tests, therefore, it is necessary to set up appropriate rigid control mixtures containing normal serum and the virus containing sap.

van Slogteren, E. (1952) and others pointed out that the precipitation reaction demands too great a quantity of antiserum which is further complicated by the serial dilutions and the use of a water-bath. Several

modifications of the precipitation reaction have been adapted, however, to facilitate rapid field analysis which will be discussed later.

(2) *Complement Fixation*

It is complicated, laborious and not often used in work concerning plant viruses (Bawden, 1950; Matthews, 1957). The complement fixation method first used for potato virus 'X' (Spooner and Bawden, 1935) was recently modified by Moorehead and Price (1953) for tobacco mosaic virus. The combination of an antigen and an antibody may be detected by means of a visible indicator system, wherein the complement (a thermolabile non-specific constituent of normal serum) is either used up or fixed.

An anti-sheep cell serum is obtained by injecting sheep red cells into the rabbit, this anti-sheep cell serum freed of its complement by heating to 56° C. for 30 min. is called *haemolytic amboceptor* (H.I.B.), or *haemolysin*. The anti-sheep cell antiserum, together with the complement, will cause the lysis of sheep red cells in the mixture. The antiserum under test is freed of any complement content by heating as indicated above. The standardised complement used in tests is prepared from fresh guinea-pig serum. The fixation experiment is carried out in two stages: (1) In the presence of a constant amount of standardised guinea-pig complement the antigen and antiserum are mixed at the various desired dilutions. These mixtures are incubated for 1 hour at 37° C. to allow the complement to be fixed by any antigen-antibody combination that may occur. (2) The indicator system is added to detect any complement that has been fixed. Washed sheep red blood cells and the heated anti-sheep cell serum are added and the mixture incubated at 37° C. Readings of the amount of lysis of blood cells are taken at various intervals.

For fixation of the complement, the reactants are allowed to stand overnight at 4° C. Haemolysin and red blood cells are then added and further incubated at 37° C. for 1 hour, before reading is taken. When the complement is fixed, no lysis will occur, while where none has been fixed, lysis will be complete. Thus, a simplified representation will be:

(*a*) Haemolytic amboceptor + Complement + Corpuscles = Lysis.

(*b*) Antigen + Antibody + Complement = Fixation.

(*c*) Antigen + Antibody + Complement + H.I.B. + Corpuscles = No lysis.

It is generally considered that complement fixation reaction is relatively a more sensitive and delicate test than the precipitation reaction for plant viruses (Bawden, 1950; Matthews, 1957). However, some differences are noticed with rod-shaped tobacco mosaic virus and the spherical turnip yellow mosaic virus (Matthews, 1957). The latter reacted more definitely than the former. This suggests that for spherical

viruses complement fixation may be useful to detect somewhat smaller amounts of virus. In light of this it would be interesting to attempt complement fixation for the spherical cucumber mosaic virus (Badami, 1958) which occurs in low concentration in tissues (Bawden, 1950). However, recent report of antisera to cucumber mosaic virus (Tomlinson, Shepherd and Walker, 1959) may pave way for an early solution of the problem of relationship of the virus with its other strains as well as with the tomato aspermy virus.

The Pennsylvania State University developed a rapid test for plant virus detection. The new method for early detection of plant viruses takes only 45 min. instead of 3–7 days as required previously and is very encouraging. The procedure is as follows: A 92% suspension of red blood cells is added to the juice of extracts from leaf or fruit tissue of plant, the clumping of red blood cells indicating the virus infection. The test has such great sensitivity that it has shown up virus infection in very early stages.

(3) *Anaphylaxis*

This is the test in which the union between antigen and antibody is detected by reactions in animal tissues. An animal that has been previously infected with an antigen may react to a second dose of the same antigen with symptoms known generally as anaphylactic shock, to which the guinea-pigs are particularly sensitive. This method is very rarely used for plant virus work.

The method consists of immunising virgin female guinea-pigs (not weighing more than 125–150 gm., for passive, 250–300 gm.) by injecting the antigen, for active sensitization. After the customary incubation period (three weeks active, twenty-four hours passive) the animals are killed and the two horns of the uterus removed. To each end of horn a thread is attached and afterwards the uterine horns are placed in aerated Ringer's solution kept at 37° C. The lower end is tied rigidly to the bath while the upper end is connected to a Kymograph needle. A small quantity of antigen is introduced in the Ringer's solution. A positive reaction is shown by relaxation and contraction of the uterine horn. Chester (1936 *a*) used anaphylaxis to distinguish between antigenic constituents of healthy and virus-infected plants. Many plants, for example, members of Solanaceae, contain substances causing non-specific contractions. However, Chester found that these toxic substances can be removed from plant extracts by a few hours' dialysis against Ringer's solution.

(4) *Neutralization of Infectivity*

This self-explanatory term implies that when viruses are mixed with normal antisera prepared against them, infectivity decreases. Under suitable conditions it can be shown that homologous antisera reduce the infectivity more than do normal sera or antisera against unrelated viruses. The most detailed work on inhibition of infectivity was done by Chester (1934) and Kassanis (1943).

Chester (1934) stated that specific and non-specific effects of antiserum are produced differently. He claims that non-specific effect decreases the susceptibility of the host plant whereas the specific one acts directly on the virus.

Kassanis (1943) states that in tests with tobacco mosaic virus, tomato bushy stunt virus and tobacco necrosis viruses qualitative differences did not exist between the neutralizing action of homologous and of other sera. Heterologous sera reduced the infectivity more than the normal sera. The specific neutralization by homologous antisera could be used for demonstrating serological relationship only if sera of the same age under identical storage conditions were compared. The unspecific neutralization of infectivity by freshly prepared sera was so large that the specific effects of homologous action of the sera decreased rapidly unless the sera were kept frozen, and in old sera the specific action predominated. The union between the viruses and their antibodies results in the loss of the characteristic properties of both, neither being destroyed. The mixtures of virus and antiserum or mixtures of virus and normal serum, form a loose non-infective complex, for on dilution, largely with saline they regain some of their infectivity. By appropriate treatments it is possible to recover both with their original properties.

Application of Serological Methods

The chief features of serological reactions are their great specificity and their great sensitivity. The serological diagnosis of plant viruses is independent of symptoms and, therefore, objective and rapid reliable tests could be carried out (Bawden, 1950; van Slogteren, E., 1955) on them. The identification of plant viruses by studying the host range and properties *in vitro* is both time-consuming and uncertain, whereas serological methods afford unequivocal results within a few minutes.

The great specificity, simplicity and greater applicability of the precipitation reaction have proved this test to be the most generally useful serological method for dealing with plant viruses. The scientific deductions are thus reduced to field practices chiefly to meet necessities, while convenient modifications or adaptations of serological methods are noticed in routine testing for virus infection, either under field conditions, mass-scale analysis, or in the laboratory in the diagnosis of disease. Agglutination tests, one of the serological methods, has been found to be an invaluable asset in large-scale field testing of potatoes for the presence of potato virus 'X'.

(1) *The Ring Test*

This is a modification of the precipitation reaction wherein the two constituents, the antigen and antibody, are overlayered one upon the other, is carried out in a narrow bore tube of 0·3 mm. diameter with the antiserum at the bottom and the antigen above. At the junction of the two, a ring or a disc of the precipitate is formed which is visible

to the naked eye. The ring test is read as a rule at twenty-minute intervals for 1–1½ hours at room temperature. The precipitation reaction is sometimes referred to as the flocculation test also.

Micro-precipitation test.—This, developed by van Slogteren, D. H. M. (1954 *b*), is a novel and notable modification of the precipitation reaction and minimises the volume of the antiserum used. The micro-precipitation method consists of covering the bottom of Petri-dishes (10 cm. diameter having no visible flaws) with a hydrophobic transparent film of some plastic preparation. The preparation of 1% Formvar (polyvinyl formal) or a substitute backed on a coating of silicone DC 1107 would provide a permanent and washable hydrophobic surface (Matthews, 1957). The droplets of a mixture of antiserum and antigen are put on this layer, drops do not spread out or coalesce, unless mixed with a very small glass rod. The serum used should be 5 times the volume of the antigen preparation. The droplets are covered by gently pouring a layer of paraffin oil over them from one side of the dish. Between 60 and 100 reactions could be carried out in one Petri-dish, which saves not only time and serum but also much space in the incubator. The precipitin reaction can be observed under normal or better still, under a dark field microscope.

(2) *Gel Diffusion or Agar-Gel Method*

This method was developed by Ouchterlony (1949) and used for plant viruses by van Slogteren, D. M. H. (1954 *a*). The method is based on the diffusion towards each other of antigen and antibodies in agar or gelatin gels and the formation of precipitation lines. He describes the modification of the gel diffusion method in which agar is poured in Petri-dishes using a metal matrix to produce several peripheral and one central cavity in the agar. Antiserum is placed in the central cavity and virus-containing preparations in cavities towards the edges of the Petri-dishes. After standing for 2–6 days, a zone of specific precipitate may form in the region between the cavities containing the virus and the antiserum. The interpretation of results presents some difficulties, since multiple zones of precipitation may be produced by effects other than the presence of a multiplicity of antigens. This technique is more suitable for spherical plant viruses. Kleczkowski (1957) using the gel diffusion precipitin test for tobacco mosaic virus separated the antigens by absorbing with antiserum. Mansi (1958) modified the plate gel diffusion to slide gel diffusion precipitin test and carried out tests on microscope slides.

(3) *Agglutination Test*

The precipitation test being more complicated and cumbersome, it necessitates search for a simpler suitable method for routine identification of viruses in the field by any non-specialist, and this resulted in the development of the agglutination test, a method devised by Chester (1937 *a*). In this simple field test, a piece of the leaf tissue to be tested, wadded into a ball, is covered with a square piece of cheese cloth and crushed by pressing between fingers so that enough crude juice to fill

up to 2 ml. level mark scratched on a 5 ml. Wasserman's tube is obtained. The rest of the tube (*i.e.*, 3 ml. up to the top) is filled with diluted serum and the two are mixed by shaking and set on the ground. A positive reaction is indicated after a few minutes by the appearance of a flaky green precipitate which can best be viewed in transmitted light or by using a flash-light. The principle is the same in both precipitin reaction and agglutination test. In the precipitin reaction the antigen (virus) material is in solution, while in agglutination it is in suspension. Sometimes a spontaneous precipitate occurs mainly if old and yellow leaves are used. The advantages of agglutination test is the possibility of use of crude infective sap in reactions.

van Slogteren, E. (1945) found a modification of this method as an indispensable tool for rapid testing of potato stocks for the presence of potato virus ' X '. This slide agglutination test is very much similar to agglutination tests described above. It uses a drop of crude infectious plant sap which is mixed with the diluted antiserum of the virus on a microscope slide, when the clumping together of small particles of host materials of plastids (chiefly chloroplasts) results in agglutination. This is observed with the unaided eye, or, if necessary, with a low powered microscope. In these tests, no antibodies specific for the plant material are involved.

In Holland, suitable handy tools devised for crushing the tissues to extract small volumes of sap are in vogue. This invaluable technique aids the rapid testing of a large number of plants in a short time. van Slogteren, E. (1952) mentions that the slide agglutination test is applicable when antisera have a high titre and are antigenically very sensitive as in the potato virus 'X', where horse antisera are used for detection of presence of potato virus 'X' (van Slogteren, E. 1955). In Holland, every year more than a million mother plants are serologically tested for production of healthy seed potatoes. One of the substations had attained a capacity of testing 21,000 samples a day and all the testing is done in a period of 3–4 weeks before lifting; this ensures the healthiness of stock of potatoes. Bawden *et al.* (1948) suggested that testing of potatoes for the presence of potato virus 'X' need not be delayed until plants have foliage; instead, the sprouts supply a reliable source of material for both serological and infectivity tests, which could be made during winter from sprouted potato tubers. This helps in discarding the infected seed tubers and allows the sowing of healthy ones. Matthews (1957), using agglutination test for the turnip yellow mosaic virus in Chinese cabbage, suggested that it could be used for all strongly antigenic viruses such as tobacco mosaic virus, potato virus 'S', etc.

Numerous modifications of the slide agglutination tests have been described. These have been made chiefly to reduce the manipulations involved, so that tests could be done in field by farm workers, or by persons with a minimal amount of training.

In Germany, Stapp and Bercks (1948) used wafers of paper impregnated with potato virus 'X' antiserum as a simple method of distributing antiserum to the workers on potato breeding farms. Potato virus 'X'

antiserum is spread on thin white paper and dried in a desiccator at room temperature over calcium chloride. Wafers 4 mm. square are cut from the dried sheets and from sheet similarly treated with normal serum. To make a test, a wafer of antiserum paper and one of normal serum are placed one at each end of a microscope slide. To each paper a drop of 0·9% saline is added, followed by a drop of the sap to be tested, the sap being first partially clarified by low-speed centrifugation. The slide is incubated at 23° C. for 20 min. then examined under a microscope in a dark field at a magnification of 50 times for the precipitation reaction.

In Canada, tests for potato virus 'X' in fields are done by an even simpler procedure (Bradley, 1952; Munro, 1954) which is partly based on the methods of Chester (1937 *a*) and van Slogteren, E. (1945). The method described is useful for routine identification of viruses in the field by the non-specialist.

(4) *The Slide Test*

A drop of sap from a potato leaflet is squeezed on to each end of a microscope slide. A drop of suitably diluted potato virus 'X' antiserum is added to one drop of sap and a drop of normal serum to the other. The drops are stirred with the opposite ends of a wooden tooth pick which is then discarded. Agglutination of sap from an infected plant usually occurs within about 10 seconds of stirring. The stirred droplets on the slide are held for examination at the junction of a partly covered light source (a mirror used in the field tests). The agglutination can then be clearly seen without magnification. Using this method, 4 men can carry out about 800 tests a day, over long periods, including collection of samples. This method is somewhat less reliable than the German and Dutch methods, for 20% of plants found infected by inoculation to *Datura* gave negative results on slide test, this discrepancy being most probably due to a lower concentration of virus in plants.

Bradley (1952) using a similar method found that a visible precipitate could be seen easily by the naked eye by holding slides against white or fluorescent tube-light or against a black-board. The great advantage is that the tests are carried out in the field very rapidly, about 125 plants per hour. He used wooden stake with clothes-pin for holding the glass slides, while the reaction was in progress.

Thus, the chief value of the agglutination test lies in its simplicity, requiring no prior treatment of infective crude sap and a minimum amount of antiserum and, above all, its great adaptability for use in the fields. The limitations of this technique are spontaneous agglutination, less sensitivity of reaction and its restricted application to highly antigenic viruses occurring in high concentration.

(5) *Group Testing*

The method of group testing was described by Markham *et al.* (1948) for potato virus 'X'. This test is applicable when a number of

plants in large fields are to be tested for freedom from a virus which occurs in obviously systemically infected plants and saves much time and labour in that a single test on leaves taken from several plants can be made. The leaves from one fully infected potato plant are mixed with an approximately equal amount of leaf from each of nineteen virus-free plants for convenience; the resulting mixed sap will give a good specific precipitate for the precipitation end-point of this virus in potato leaf sap is normally at least 1/1000. In group testing method it is important to collect the same amount of leaf from each plant. Otherwise the sap from diseased plants may become much more diluted. Very little antiserum is used in group testing. One hundred ml. of an antiserum used in 0·5 ml. quantity at 1/20 dilution to test groups of 10 plants, would test 40,000 plants. However, individual diseased plants cannot be identified by this method. An estimate of the percentage of infected plant can be obtained from simple statistical analysis, provided the diseased plants are few. A more detailed account of group testing is given by Matthews (1957).

The precipitation reaction has been widely used to determine:

(i) (*a*) Relationships and classifications of plant viruses. (*b*) Relationships and classifications of bacteria.

(ii) (*a*) Shape of bacteria. (*b*) Shape of virus particles.

(6) (*a*) *Relationships and Classifications of Plant Viruses*

In India, Desai (1935) working with the sugarcane mosaic virus attempted to establish relationships between the organism associated with sugarcane mosaic virus and the filterable forms, using serological tests. He found that complement fixation was not sensitive enough but sufficient to show relationship between the virus, filterable form and the organism, this relationship being supported by results of the agglutination test, which was more definite. Chester (1937 *b*) demonstrated the value of serological tests as a basis for relationship and classification of plant viruses and, by applying the test to 40 types or strains of viruses, recognised clearly distinct groups. Later, Bawden (1950) strongly advocated serological relationships as chief criteria for an ideal system of classification of plant viruses. He drew up a list of viruses related in this manner and recognised 17 groups (Table I).

This table is partly based on Chester's (1937 *b*) results. There is little doubt that serology can be more widely used for showing relationships than it has been possible so far and it could be presumably extended beyond the seventeen antigenically distinct types that have been identified, each of which may be regarded as a separate species.

Cucumber viruses 3 and 4 have certain antigen in common with tobacco mosaic virus (Bawden and Pirie, 1937 *b*) as shown by serological tests. This relationship could not have been determined by cross-immunity (plant protection) test on host plants, because these (cucumber viruses and tobacco mosaic virus) have no common host plant. Tobacco necrosis virus group is in reality a number of viruses biologi-

TABLE I

Viruses grouped by serological relations

(After Bawden, 1950)

The viruses within each group precipitate with each other's antisera, whereas those in different groups do not.

Group	
1. *Tobacco mosaic*	.. Common and masked tobacco mosaic viruses; tomato aucuba mosaic; enation mosaic and streak viruses; Japanese petunia virus; Holmes' rib-grass virus; cucumber viruses 3 and 4.
2. *Potato X*	.. Salaman's H, G, L, S and N strains; potato viruses B and D, potato mottle and ring-spot viruses; Hyoscyamus virus 4.
3. *Potato Y*	.. Potato leaf-drop streak; rugose mosaic and veinbanding viruses; potato virus C; tobacco veinal necrosis virus; hyoscyamus virus 2.
4. *Tobacco etch*	.. Severe and mild etch viruses; Blakeslee's Z-mosaic virus of *Datura*.
5. *Henbane mosaic*	.. Severe and mild strains of hyoscyamus virus 3.
6. *Cucumber mosaic*	.. Price's isolates of cucumber virus 1; Valleau's delphinium virus.
7. *Soya bean mosaic*	
8. *Pea mosaic*	.. Osborn's pea viruses 2 and 3.
9. *Tulip mosaic*	
10. *Sugar-beet yellows*	
11. *Tobacco ring-spot*	.. Wingard's and yellow and green tobacco ring-spot virus; tobacco ring-spot virus 2.
12. *Tobacco necrosis* A	.. Potato, Princeton and tobacco VI cultures.
13. *Tobacco necrosis B*	.. Tobacco I and II cultures.
14. *Tobacco necrosis C*	.. Rothamsted cultures and bean stipple-streak.
15. *Tomato bushy stunt*	
16. *Southern bean mosaic*	
17. *Turnip yellow mosaic*	

cally similar, but serologically unrelated. This could not have been demonstrated by the symptomatology on the host plants (Smith and Bald, 1935; Bawden, 1941).

Serological methods do not identify viruses precisely, for reactions are only group specific. With the antiserum to tobacco mosaic virus, if a sap is specifically precipitated, it is just an indication of the presence of a strain of this virus, but not the identity of the particular strain.

In order to differentiate between virus strains it is necessary to use the absorption technique, clearly explained by Bawden (1950) and Matthews (1957). For three strains of the same virus, represented by small letters for determinant antigens (virus) and their respective antisera indicated by capital letters for corresponding antibodies, the whole formulae will be:

Antigenic grouping	Antibodies
Strain 1: a, b, c, d, e	Antiserum 1: A, B, C, D, E
Strain 2: c, d, e, f, g, h	Antiserum 2: C, D, E, F, G, H
Strain 3: a, b, g, h, j, k	Antiserum 3: A, B, G, H, J, K

Since each antiserum contains antibodies in common, the mixture of any strain with any antiserum will bring precipitation of virus; the effect of antiserum depends on the strain mixed with it. Thus,

Strain 1	a b c d e
Antiserum 1	A B C D E

when mixed, all 5 antibodies will be absorbed and removed in precipitate, the supernatant fluid incapable of further precipitating action. However, if strain 2 is mixed with antiserum 1:

Strain 2	c d e f g h
Antiserum 1	A B C D E

= the supernatant will contain the antibody A B. Similarly, combinations are possible.

Chester (1936 *b*) used this absorption technique to differentiate between 10 strains of tobacco mosaic virus and 3 strains of potato virus 'X'. Bawden and Pirie (1937 *b*) using cross-absorption techniques with various strains of tobacco mosaic virus and their antisera, also showed that cucumber viruses 3 and 4 are related to tobacco mosaic virus. Matthews (1948) used this method with 10 strains of potato virus 'X', 6 of them being found indistinguishable by the method used.

In using the cross-absorption test for identifying virus strains a " mirror-test " can be conducted (Bawden, 1950). This has to be done before two strains can be shown to be antigenically identical. Antisera are prepared separately against each and it can then be shown that neither reacts with its homologous antiserum after absorption with the other strain. Matthews (1957) points out certain limitations of cross-absorption procedure:

(1) Dilution of serum by heterologous antigen preparation;

(2) The effect of titre of antiserum on the specificity; and

(3) Dependence of the number of possible fractions on the number of strains tested. Two strains with the same antigen in different proportions will interfere with cross-protection.

Serological techniques have now been successfully applied to about 15 different plant viruses, but they have failed with many others (Bawden, 1950). Most of the serological work has been carried out for obvious reasons on sap-transmissible viruses. A few attempts made to prepare antisera against non-mechanically transmissible viruses with specific insect vectors (Smith, 1951) have not so far yielded any result.

(6) (*b*) *Relationships and Classifications of Bacteria*

There is practically no work done in India, except for using of serological methods in an attempt to establish relationship among some pathogenic bacteria (Patel *et al.*, 1951).

The notable work done in India is an attempt to establish serological relationships among species of phytopathogenic bacteria (Patel *et al.*, 1951). They used serological agglutination test to distinguish the different species of *Xanthomonas*. The eight organisms tested were placed in five groups showing closer definite relationships amongst the species.

(1) *X. desmodii, X. cassiae, and X. alfalfae,*

(2) *X. campestris,*

(3) *X. vignicola and X. vesicatoria,*

(4) *X. desmodii-gangeticii,*

(5) *X. malvacerum.*

Xanthomonas translucens, the causal agent of bacterial stripe blight of cereals and grasses, showed diversity in pathogenicity (Fang, Allen, Riker and Dickson, 1950). From pathogenicity studies of *X. translucens* from cereals, grasses were differentiated into five special forms (*formae speciales*) as follows: Hordei (barley,) Undulosa (wheat), Secalis (rye), Cerealis (smooth brome grass and quack grass) and Phleipratensis (timothy). Some gummy substance produced by the organism seemed to interfere with the antigenic pattern of organisms. There was lack of differentiation amongst the first four *formae speciales* by their cross-agglutination reaction results. Nevertheless, it was

possible by agglutination absorption test to recognise four serological types: (*a*) Hordei, (*b*) Undulosa and Secalis, (*c*) Cerealis, (*d*) Phleipratensis.

Mushin, Naylor and Lavhovary (1959), using high-titre antisera, attempted to determine serological relationships amongst 15 species of plant pathogenic bacteria belonging to the genera *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. In tests they used slide and tube agglutination and agglutination absorption methods. Further, they concluded " that serology cannot replace other bacteriological techniques but it can be very helpful as an additional tool for characterisation of species or strains of plant pathogenic bacteria. Serology may be useful in epidemiological studies, in tracing the spread of infection and as a rapid means of identification of isolates in plant pathogenicity tests ".

Shattock (1955) strongly urges the use of serology in the classification of micro-organisms, for according to him the use of serology can give clue to the possibility of close natural relationships between *Staphylococci*, *Streptococci* and *Pneumococci*. The identification of Enterobacteriaceae (*Salmonella*) is widely done by the agglutination tests. The diagnosis of species in *Lactobacillus* by serological techniques has been notoriously a difficult problem and has helped little to clarify the situation.

Bacteriophages (Bacterial viruses).—Some workers (Delbrück, 1946; Adams, 1953) have emphasised serological criteria as the first and the most important for classification of bacteriophages. The seven phages of T-series of coliphages grouped on serological basis fell into four distinct and unrelated serological groups in agreement with morphological differences (Delbrück, 1946).

(7) (*a*) *Shape of Bacteria*

Topley and Wilson (1946). cited that in agglutination tests, the thermolabile flagellate bacterial antigens on mixing with their antisera, are agglutinated and form large clumps of fluffy open structure referred to as Hauch form, also known as " *H-type* ". The thermostable bacterial antigens are agglutinated more slowly and form smaller clumps which are dense and granular, referred to as Ohne form or " *O-type* ".

(7) (*b*) *Shape of Virus Particles*

Essentially similar differences are found between the form of precipitate produced by different viruses with their antisera, which will give reasonable clue to the particle shape of viruses. Bawden (1950), using this character of specific precipitate, has shown that the flocculent, open, fluffy, H-type precipitate indicated the rod-shaped virus particles exemplified by tobacco mosaic virus, potato viruses 'X' and 'Y'; while a dense granular O-type precipitate suggests the spherical plant viruses exemplified by tobacco necrosis virus, turnip yellow mosaic virus and lucerne mosaic virus. Thus, Matthews (1957) records 14 elongated viruses and 13 spherical viruses.

(8) *Fungal Serology*

Serological methods have only been casually used in diagnosis of fungus diseases. In this brief account some of the important genera of fungi, for which serological methods have been employed is presented (Table II). For further details, reviews of Link and Wilcox (1933) and Tempel (1959) may be consulted.

TABLE II

Showing the genera of fungi for which serology has been applied

Genera	Authors with year
1. Representatives of algae and fungi ..	Steinecke, 1925.
2. Phytopathogenic Ascomycetes and fungi imperfecti	Link and Wilcox, 1933.*
3. Basidiomycetes: relationships (*a*) cereal rusts—*Puccinia* (*b*) Smuts—*Ustilago*	Neuhoff and Ziegenspeck, 1926. Bahn, 1957, Mamontova, 1954. Beck, 1934; 1938.
4. *Fusarium* ..	Coons and Strong, 1928. Link and Wilcox, 1933. Tempel, 1957, 1959.
5. *Aspergilli* ..	Matsumoto, 1928, 1929.
6. Yeasts in brewing ..	Schutze, 1903.

* The precipitin ring test is widely applied to fungi in general.

Antigenic properties of phytopathogenic fungi

Fusarium.—The genus *Fusarium* which is taxonomically one of the most confusing genera of the fungi imperfecti and phytopathogenically the most important division of the Phragmosporae (Link and Wilcox, 1933) shows antigenic properties. The identification and classification of the organisms considered as species of this genus are often difficult because when grown on artificial media, they invariably fail to produce distinguishing morphological structures—the conidia. Some members of this genus have been studied widely employing serological methods. Thus, Coons and Strong (1928) using complement fixation technique were able to differentiate between the closely related species of *Fusarium radicicola*, *F. conglutinans*, *F. martii* (var.) *phaseoli*, *F. oxysporum* and *F. orthoceros*. Later, Link and Wilcox (1932, 1933) first began work with the three species, *viz.*, *F. conglutinans*, *F. cubense* and *F. lycopersici*,

but later included 19 more species or strains of fungi, like, *Cylindrocarpon album* (Sacc.) Wr., *Ramularia*, *Neurospora* (= Monilia) *tetrasperma* Shear and Dodge, *Sclerotinia fructicola* and *S. sclerotiorum*, for serological studies. They employed precipitin ring tests in their work. Tempel (1957, 1959), in extensive studies with *Fusarium vasinfectum*, suggested that it might be possible to apply sero-diagnostic methods in distinguishing *formae speciales* and physiologic races. He used gel diffusion precipitin tests to distinguish between *F. oxysporum* f. *lupini* (Linf) Sn. *et* H. and *F. oxysporum* f. *pisi* (Linf) Sn. *et* H. species. He also found serological relationships between *Polyspora lini* and *Pullularia pullulans*, differing in one factor (Tempel, 1958). Fedotova (1938 *a*, 1939) by the intensity of serological reaction could fairly and accurately estimate the resistance or susceptibility of cotton varieties to the attack by *Verticillium dahliae*, *F. buharicum* and *Bacterium malvacearum*. Serological methods have been extended to cereal rusts (*Puccinia triticina*) by Fedotova (1938 *b*) and with other *Puccinia* species, like *P. graminis tritici*, *P. graminis avenae* and *P. sorghi* (Bahn, 1957). Closely related members of the Ustilaginaceae were differentiated by applying serological techniques (Beck, 1934, 1938). He used standard precipitin ring test to differentiate smut fungi: (1) Monosporidial cultures of *Sorosporium reilianum*, *Ustilago hordei*, *U. levis*, *U. zeae*. (2) Mass cultures of *U. hypodites*, *U. tritici*, *U. avenae*, *U. levis* and *U. zeae*. The former could be satisfactorily differentiated, while the latter could not as they showed many cross-reactions. Mamontova (1954) using a spore suspension of wheat loose smut (*U. tritici*) obtained a highly active specific serum. She also claimed positive reactions between the serum and antigens of susceptible wheat varieties.

Nature of the antigens.—Tempel (1959) discussed that the antigens in culture liquids are partly resistant to heating at 60° C. for one hour and partly to heating at 100° C. for one hour. The reaction of antisera against culture liquids and mycelial extracts appeared to be weaker with liquids heated to 60° C. than with untreated liquids. Rabbits produced antibodies against liquids heated to 60° C. but not against the liquids heated to 100° C. Tempel (*loc. cit.*) suggested that " in the mycelium glycoproteins are present and that these glycoproteins split up into polysaccharides and various proteins during autolysis in the cultures and during extraction of the mycelium. Rabbits only produce antibodies against proteins after injection with culture liquids or with saline extracts of mycelium. The antibodies for polysaccharides can be obtained only by immunising with microconidia. These polysaccharides are probably present at the surface of the cell-wall of the microconidia".

Methods in fungal serology.—Link and Wilcox (1933) found Richard's solution* suitable (protein-free liquid medium) for *Fusarium conglutinans*, *F. lycopersici*, *F. cubense*, which grew in sufficient abundance. Cultures were grown in 100 ml. of solution in 250 ml. Erlen-

* Richard's solution: 33·3 gm. sucrose, 6·6 gm. KNO_3, 3·3 gm. KH_2PO_4 and 1·7 gm. $MgSO_4$ in 1,000 ml. distilled water.

meyer flask by inoculating from a seeding flask (inoculated 4–5 days previously) containing a cloud of spores and hyphal fragments well dispersed by shaking. After one day's shaking the culture flask was left undisturbed in subdued light at 20–30° C. for 3–4 weeks, to facilitate good mat production. The dry weight of the material ranged from 20 mg. to approximately 80 mg. They also gave details of the recovery of fungus mats, pulverisation of mats, and extraction of powders. The lipoid-free powder was thoroughly dried preliminary to saline extraction, which treatment tended to enhance the antigenic properties (of *F. cubense*, *F. lycopersici* and *F. conglutinans*) of injected material and the reactive properties of the test antigens.

The standard preparation of the antigens followed by Link and Wilcox (1933) was as follows: for the test antigen 1 : 50 stock dilution, 0·3 gm. of powder was ground with pumice (3 minutes) extracted with petrol ether until fat free, dried and extracted in 15 ml. of 0·85% saline solution for 18 hours at 0° C. After centrifugation sparkling clear extracts were obtained. Antigens for injection were extracted directly in saline from these fungal powders without preliminary pumice and petrol ether treatment. All antigens were stored at 0° C. Every 2 weeks, fresh lots of test antigens were usually prepared.

Immunisation.—Intravenous injections of either clear extracts or suspended fungal material failed to produce antisera because in the former the titres were too low and in the latter the animals quickly succumbed to embolism. A combination of intravenous injection of a clear saline extract of the powder and intraperitoneal injection of the extracted powder resuspended in saline was found to give adequate titres. After a 5-day rest to the animal the desired number of series of injections was continued. After the last injection was given the animal was taken off feed the fourth day and bled the fifth day. Forty ml. blood furnished sufficient serum for tests against 20–25 antigens. No preservatives were added to the antisera which were obtained aseptically and stored at 0° C. with relatively slight loss by spoilage.

The precipitin tests were done in tubes of 5 mm. with mixtures of antigen and antiserum, incubated at room temperature (28–32° C.) and read at 1–2 hour intervals. The test did not readily differentiate all entities, which are separable by morphological and physiological criteria, such as host and symptom specificity. Each organism appeared as a distinct serological entity. The authors admit that results obtained with absorption of precipitins were inconclusive because of the unreliability of the technique used. Recently Tempel (1959) greatly improved the methods by using gel diffusion precipitin tests for reactions. A number of *formae speciales* of *Fusarium oxysporum* Sn. *et* H. were studied by this method:

Fusarium oxysporum f. *callistephi* (Beach) Sn. *et* H.

Fusarium oxysporum f. *dianthi* (Prill. *et* Del) Sn. *et* H.

Fusarium oxysporum f. *upini* Sn. *e* H.

Fusarium oxysporum f. *narcissi* (cke. *et* Mass) Sn. *et* H.

Fusarium oxysporum f. *pisi* (Lindf) Sn. *et* H.

Fusarium oxysporum Schl. emend. Sn. *et* H. geisoleerd van tulp.

The fungus was cultured in Richard's medium for 10–14 days at 27° C. to obtain culture liquids and mycelium. Shake cultures were prepared in Czapek's medium to obtain microconidia. One to two-year-old rabbits were injected intravenously, intraperitoneally, subcutaneously or intramuscularly with different preparations of the fungus giving in all four injections a week. The serum was studied by means of micro-agglutination tests, micro-precipitation tests (van Slogteren, D. H. M., 1954 *b*), gel diffusion precipitin tests (Ouchterlony, 1949) and the immuno-electrophoretic method (Grabar and Williams, 1953).

The culture liquids, homogenised mycelium in saline, mycelial extracts and microconidial suspensions appeared to be antigenic. Rabbits were immunised with a homogenised culture of *F. oxysporum*. The culture liquids, mycelial extracts and sera against different preparations were compared by gel diffusion precipitin tests. Microconidia agglutinated very easily with highly diluted antisera (1 : 4096) but also with highly diluted normal serum, so that agglutination reactions could not be applied to differentiate *formae speciales*. The gel diffusion precipitin test was the most suitable to differentiate *formae speciales*. However, sera did not differentiate *formae speciales* specifically.

The methods, if fully explored, may prove very profitable particularly for establishing the serological relationships (based on serology) between the different races of obligate parasites.

(9) *Detection of Unknown, Latent or New Viruses*

De Bruyn Ouboter (1951) used a potato variety ' Light Industrie ' as the source of inoculum of potato virus ' A ', for the preparation of an antiserum against it, and obtained an antiserum which reacted positively with an antigen, which could not be potato virus ' A ', as revealed by the symptomatological picture and electron microscopic studies. Hence the presence of another unknown virus was doubted. The antiserum from ' Light Industrie ' reacted positively with the sap of Bintje potato showing faint virus-like symptoms. Later Rozendaal (1952, 1954) proved the presence of a transmissible virus by tuber plug grafting and sap inoculation. For this latent virus he proposed the name virus ' S ', in honour of Prof. van Slogteren.

This accidental discovery of a latent new virus was followed by the independent finding of carnation latent virus by Kassanis (1954). Using serological tests he was able to detect the presence of a carnation latent virus which was masked by interference of other viruses. Further, Kassanis' (1956) discovery of the unequivocal serological relationship between paracrinkle, potato virus ' S ' and carnation latent virus, helped to group them as strains of the same virus. He used the precipitation reaction to demonstrate that the three viruses are serologically related but far from being antigenically identical. This dis-

covery explained the longstanding puzzle of universal occurrence of paracrinkle virus in King Edward Potatoes.

A latent form of potato virus 'X' common in potatoes is confirmed by agglutination test, as the presence of the virus cannot be detected by inoculating on to the test plants.

Serological methods, chiefly the precipitation reaction, are useful in separating mixtures of viruses into their constituents. Badami and Kassanis (1959) availed of the precipitation reaction as one of the methods to resolve the mixture of viruses affecting *Solanum jasminoides* Paxt. From this plant three mechanically transmissible viruses were isolated. One is a strain of potato virus 'Y', which was precipitated from the mixture by its antiserum, leaving the supernatant with tobacco wilt virus and Datura necrosis virus. These two were also separated later using their respective antisera.

Serological Methods for Quantitative Plant Virus Studies

(*a*) *Estimation of the Virus Concentration.*—The precipitation reaction provides a rapid, convenient and quantitative method for estimating virus concentration. The reaction, described earlier, was first used for quantitative work on plant viruses by Beale (1934) for tobacco mosaic virus and later by Bawden (1935) for potato virus 'X'. The reaction being specific, the virus estimated in presence of plant impurities or of unrelated viruses does not affect the results. The greatest value of this method is that the results can usually be translated directly into relative virus concentration of samples. In the absence of suitable local lesion hosts for quantitative work with virus or strains of virus or in case of difficulty in mechanical transmission, serological methods are of particular use. Another great merit of the precipitation reaction is that antisera remain constant for appreciably long periods and tests can be made under standardised conditions and the results obtained at different times are comparable with those obtained at one time.

In spite of the many advantages of precipitin reaction most workers seems to have neglected it and have made quantitative estimations exclusively by the more laborious and less accurate local lesion method. This may be due to unfamiliarity with the technique. The precipitation reaction measures the relative concentration of serologically active virus protein, while the local lesion count inaccurately measures the relative infectivities of different preparations. However, the precipitation reaction fails not only to distinguish between infective and non-infective virus particles but also fails to distinguish related virus strains of the same virus. Nevertheless, the special value of precipitin tests for quantitative work with viruses that are difficult to transmit mechanically or that form no countable local lesions is too obvious to stress. The precipitin test, used in addition to the infectivity tests, the two combined will give not only more accurate results but also often provide more and different information than either method alone could give.

The several ways in which the precipitation reaction method can be used for quantitative work have been discussed in detail by Matthews (1957). Here, only a brief outline of the more important methods are given.

(*b*) *The Precipitation End-point.*—This is the highest dilution of antigen at which a visible precipitate is obtained after a given period of incubation with its antiserum. This is a more accurate and widely used method than optimal precipitation point (*see* below). In estimations the diseased plant sap can be used with an antiserum of fairly high titre (at least 1/16) for the determination of the virus end-point.

A series of two-fold dilutions of each virus sample to be compared is made in 0·85% or 0·14 M saline. An equal volume of the antiserum at a constant dilution is added to each precipitin tube with the diluted antigen. The tubes after shaking are incubated in a water-bath held at 37–50° C. for 2 hours. The time and nature of precipitate formation are noted and the results recorded. Chester (1936) used (+) signs to indicate the reactions,

++++ = Very strong reaction
+++ = Strong
++ = Moderate
\+ = Positive reaction but not strong
(+) = Weak reaction, questionable.

With the bushy stunt, tobacco necrosis and turnip yellow mosaic viruses, all having spherical particles of uniform size, the virus end-point test gives reproducible results. However, with rod-shaped viruses aggregating as in tobacco mosaic virus, results are difficult to interpret. More precaution is necessary in comparing two samples of a rod-shaped virus by this method, for, they should not have undergone different treatment which greatly alter the proportions of long and short particles (*e.g.*, heating, centrifugation, salt precipitation). These help them to aggregate.

(*c*) *The Optimal Precipitation Point.*—This is the ratio of antigen to antiserum at which precipitation first occurs and although this method is less accurate, it nevertheless gives results quickly. There are two different optimal precipitations: (1) *Optimum* which is the highest dilution of the virus that precipitates most rapidly with a constant dilution of the antiserum; (2) *Optimum* which is the highest dilution of antiserum which precipitates most rapidly with a constant dilution of virus. The optimum precipitation is, however, slow enough to be determined and is fairly proportional to dilution of the reagents. This is the basis of its use as a quantitative procedure. Both the methods are interchangeable and find application in virus estimations.

A series of two-fold dilution is made of virus preparations to be compared, and antisera at suitable constant dilutions are added quickly to each tube containing the virus dilutions beginning with the most

dilute. The contents of tubes are shaken, then placed half-immersed in the water-bath. The first tube showing a visible precipitate is noted. It is generally considered that a precipitation time of 20–30 min. in the fastest tube is about the ideal for quantitative reactions. This procedure is used to compare the concentration only of serologically identical or very closely related strains of a virus.

Failure of Serological Tests

There are a number of possible reasons why application of serological methods to any particular virus may fail. Matthews (1957) indicates possibilities: (i) instability of virus in plant sap, *i.e.*, rapidly becoming antigenically inert, which may be due to the pH of the expressed sap, oxidising properties of sap, plant enzyme action and thermal instability of the virus; (ii) the virus combining with some components of plant sap, making it serologically inactive; various compounds having this effect are known, *e.g.*, tannins and tannin-like substances and normal plant proteins; (iii) the virus occurs in too low a concentration in the plant sap to give detectable antigenic reactions; (iv) although stable viruses occur in sufficient concentrations they are very poorly antigenic; (v) not all plant viruses are antigenically active.

Some Problems in Plant Virus Serology

The problems posing in plant virus serology are many and varied in nature. The most prominent is the failure to apply serological methods for all plant viruses, but it is hoped that in the course of time as techniques improve this difficulty may be overcome.

The present arbitrary methods of designating the physiologic races of rusts and other obligate parasites are well known. The races of *Phytophthora infestans* are distinguished by morphological characters, and the reactions on a set of differentials are also well known. However, these are not accurate ways of identification as evidenced from the fact that an element of individual discretion in reading the results creeps in. The successful use of serological methods for arriving at more accurate relationships in the grouping of pathogenic bacteria (*Xanthomonas* spp.) and the wilt fungus (*Fusarium* spp.) is very encouraging. If serological methods are properly exploited it would be possible to achieve more concrete results and possibly a most lasting system of classification in many groups where morphology alone is the criterion used but found unsatisfactory as new mutants arise presenting a challenge to classification.

Although many Western countries use serological methods for plant virus study, in the production of healthy seed potatoes and flower bulbs in India, methods of serology have not yet found a place in the study of plant viruses or in regular practical applications to raise healthy seed potatoes. From the figures of the Tuber Crop Committee of the Indian Council of Agricultural Research it is distressing to note that only 30% (less than half) of the demands for healthy seed potatoes are

met from within the country. The importance and unlimited practical applicability of serological methods need no stressing and the early introduction of serological techniques for testing of virus diseases and selection of healthy seed potatoes would go a long way in promoting a sound agricultural economy for this country.

The alarming rapidity with which cereal viruses are being recorded, particularly the seed-borne virus disease of barley false stripe, would necessitate the discovery of an early rapid diagnosis, possibly by serological methods.

Addressing the Jubilee Proceedings of the Association of Applied Biologists, Bawden (1955) said, " I end, as I began, with the plea for more work on viruses, which now provide one of the most fascinating subjects for botanical research. So far in Britain their study has been almost the exclusive prerogative of applied biologists in research stations and research institutes. There is much to be done that would be appropriate to university botanical departments, and it is to be hoped they will join in. When we consider how much they have contributed to our knowledge of fungi and bacteria, we can be sure that, if they will now begin to take an active interest in the viruses whoever talks on the control of virus diseases at the Centenary Meeting of the Association of Applied Biologists will be able to talk more confidently and paint rosier picture than I can today". These wise observations need hardly a recommendation and doubtless, progressive university centres in India would take to study of this fascinating field of specific human endeavour. Indeed, a beginning has been made at University Botany Laboratory, Madras, and an antiserum to Dolichos enation mosaic virus (Badami, 1959) is now available for virus serologists at home and abroad. van Slogteren and van Slogteren (1957) advocated: " An international exchange of samples of antisera will not only be useful to purposes of identification, classification, and nomenclature of different viruses described in various countries, but also to the identification of different virus diseases of plants.....Without any doubt the opportunities opened by serological diagnosis of more plant virus diseases will lead to more healthy crops and will give due satisfaction to the scientists who laid the foundation of plant serological research about thirty years ago."

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* Originals not seen.

CYTOLOGICAL STUDIES IN THE MOSSES OF EASTERN INDIA

I. *Hydrogonium consanguineum* (Thw. *et* Mitt.) Hilp., *Semibarbula orientalis* (Wib.) Wijk. *et* Marg., *Vesicularia montagnei* (Bel.) Fleisch., *Taxithelium nepalense* (Schwaegr.) Broth. and *Philonotis laxissima* (C.M.) Bryol. java.

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Considerable interest is being shown of late in cytological studies involving the lower groups of plants. This has also extended to the field of Bryophytes including the mosses. Miss Wylie (1957) has enumerated chromosome numbers of 364 species worked out up to that date. The technique of studying moss chromosomes, though rather uncertain, has greatly improved in recent years. Such studies have shown that cytotaxonomical knowledge is essential for the proper understanding of the phylogeny of species as it has been definitely established that hybridisation, polyploidy and gene mutation, coupled with isolation, are important factors contributing to the evolution of new plant species.

The interesting plant group of mosses had been rather neglected in India in the immediate past. However, of late, some cytological investigations have been undertaken on Indian mosess (Banerji and Sen, 1957; Pandé and Chopra, 1957, 1958 ; Chopra 1957). The present authors are carrying on similar investigation on mosses from North-East India.

Material

Barbula Hedw. is the commonest wall and ground moss genus round about Calcutta. This genus has now been broken up into several full-fledged genera (Chen, 1940), of which only *Hydrogonium* (C. Müll). Jaeg. *et* Sauerb. and *Semibarbula* Herz. *ex* Hilp. are common here. *Semibarbula orientalis* (Web.) Wijk. *et* Marg. (= *Barbula indica* Schwaeg. *ex* Brid.) dominates the walls while *Hydrogonium consanguineum* (Thw. *et* Mitt.) Hilp (= *Barbula consanguineum* Thw. *et* Mitt.) along with a few other species are found on clay soil.

Both *Vesicularia montagnei* (Bel.) Fleisch. and *Taxithelium nepalense* (Schwaegr.) Broth. grow as epiphytes on various trees roundabout Calcutta and may extend to the soil if sufficiently moist and shady. Sterile and slender *Philonotis laxissima* (C.M.) Bryol. java. plants

are found to develop on flowerpot loam soil in greenhouses towards the end of the rainy season.

Method

The meiotic studies were made from capsules when these were just mature but still green and slightly translucent. In some cases, divisional stages were found in capsules with slightly coloured operculum. The exact stage of fixation was found by trial. The capsules were fixed for 1½ to 2 hours in acetic alcohol (1 : 3) followed by acetocarmine squash technique or they were fixed in propionic alcohol (1 : 2) followed by propionocarmine. The latter method gave better results. For the Barbuloid species (*Hydrogonium* and *Semibarbula*) pretreatment with 0·002 M 8-oxyquinoline for ½ hour at 15–17° C. yielded better results. Peak period of meiotic division was in the morning (8 to 10–30 A.M.) in these two species and in the afternoon (1 to 2–30 P.M.) in *Vesicularia* and *Taxithelium*. Very often, division was not uniform, same capsule showing both tetrads and dividing stages.

For gametophytic study (which was the only method for *Philonotis laxissima* which does not fruit in Calcutta), the very small growing tip was dissected, fixed in a mixture of 30% acetic acid and 70% alcohol (1 : 1) for 6 to 48 hours and squashed in aceto-orcein with potassium acetate (a pinch in 15 c.c. of 1% aceto-orcein). The method is principally that suggested by Lowry (1948). Stages of somatic division were more frequent in the afternoon (12–30 to 2 P.M.) in *Philonotis laxissima.*

Observations

1. *Hydrogonium consanguineum* (Thw. *et* Mitt.) Hilp.

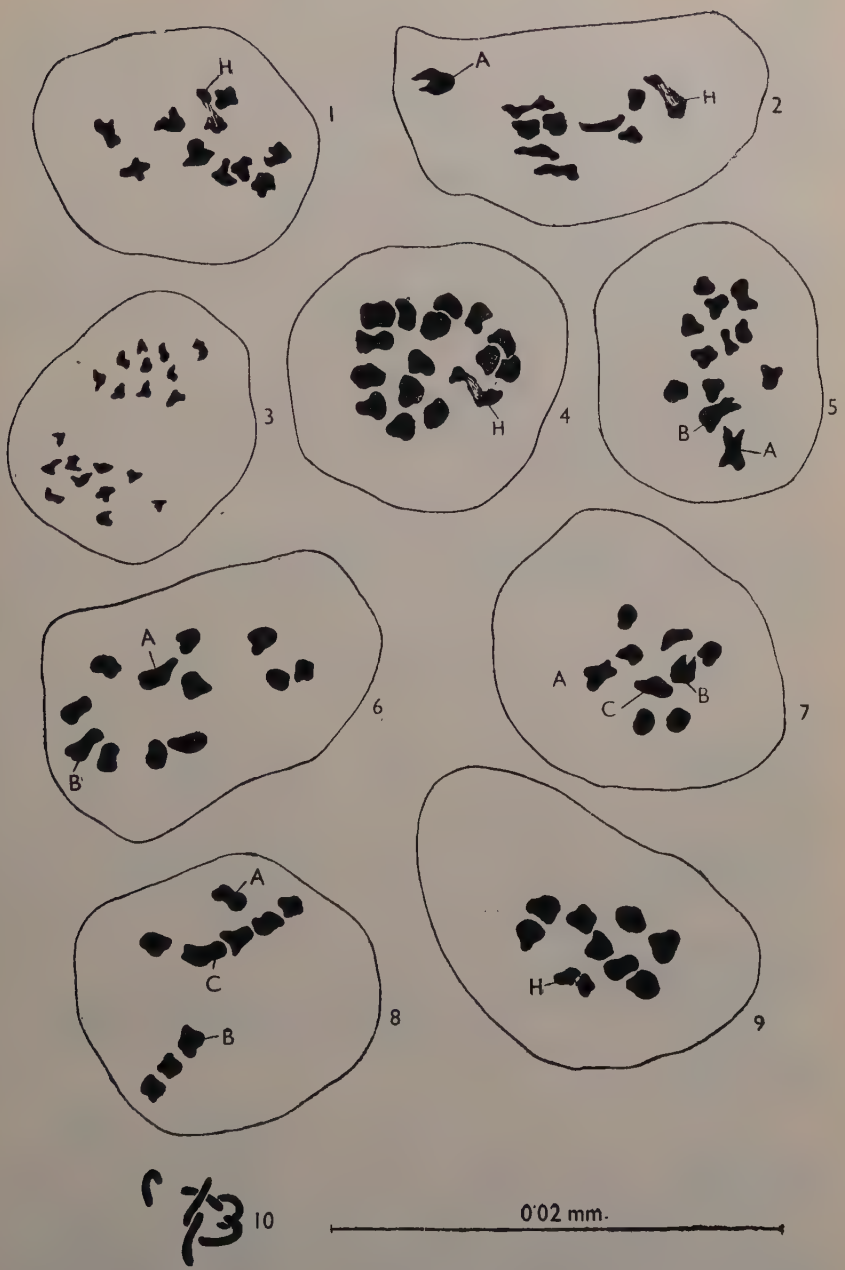
10 bivalents were counted in diakinesis (Text-Fig. 1), metaphase I (Text-Fig. 2) and 10 chromosomes in each daughter nucleus in metaphase II (Text-Fig. 3) stages. The chromosomes are so small that it is difficult to get a clear idea of their morphology but they are mostly rod-shaped and of various sizes. One chromosome pair (A) is the largest and one heteromorphic pair of chromosomes (H) is noted. At diakinesis, chiasmata at various degrees of terminalisation are noted and during anaphase certain irregularities disturbing disjunction probably cause the great variation in spore size (7 to 10·5 μ).

2. *Semibarbula orientalis* (Web.) Wijk. *et* Marg.

16 bivalents were noted during diakinesis and metaphase I (Text-Fig. 4) and the number corresponds to the finding of Banerji and Sen, 1957). One heteromorphic pair (H) was distinct. Irregularities in anaphasic separation were noted which may be correlated with the extreme variability in spore size (9·8 to 17·5 μ).

3. *Vesicularia montagnei* (Bel.) Fleisch

Capsule squashes show 12 bivalents during diakinesis (Text-Fig. 5) and metaphase I (Text-Fig. 6). The bivalents show different degrees



TEXT-FIGS. 1–10. Figs. 1–3. *Hydrogonium consanguineum*. Fig. 1. Diakinesis. Fig. 2. Metaphase I. Fig. 3. Metaphase II. Fig. 4. *Semibarbula orientalis* Metaphase I. Figs. 5–6. *Vesicularia montagnei*. Fig. 5. Diakinesis. Fig. 6. Metaphase I. Figs. 7–9. *Taxithelium nepalense*. Fig. 7. Diakinesis. Fig. 8.

Metaphase I. Fig. 9. Metaphase I showing precocious disjunction. Fig. 10. *Philonotis laxissima*. Mitotic plate.

of chiasma terminalisation and are of different sizes, 2 (A and B) being much larger than the rest, 3 comparatively small and rounded and the rest intermediate.

4. ***Taxithelium nepalense*** **(Schwaegr.) Broth.**

9 bivalents could be seen in capsule squashes during diakinesis (Text-Fig. 7) and metaphase I (Text-Fig. 8). 3 of these bivalents (A, B and C) are larger than the rest. Some of the metaphase plates show that one of the bivalents (H in Text-Fig. 9) has got the tendency to separate early so that some plates give the count of $n = 10$. Lagging of a pair of chromosomes was noted in one anaphase plate.

5. ***Philonotis laxissima*** **(C. Müll.) Bryol. java.**

Gametophytic squashes showed 6 chromosomes (Text-Fig. 10) of which 3 are rod-shaped and 3 more or less V-shaped.

Discussion

The original genus *Barbula* Hedw. (Family *Pottiaceae*) has been broken up into a number of genera by Hilpert and Chen (Chen, 1940) of which some cytological data of the following are known:

Species	Chromosome No. (n)	Author
BARBULA		
fallax Hedw. ..	9–11	Heitz, 1928
unguiculata Hedw. ..	13	Jachimsky, 1935; Vaarama, 1950
vinealis Brid. ..	14	Steere *et al.*, 1954
brachyphylla Sull. ..	12	Steere *et al.*, 1954
cylindrica (Tayl.) Schp. ..	13	Vaarama, 1953
STREBLOTRICHUM		
convolutum (Hedw.) P. Beauv. (as *Barbula convoluta* Hedw.)	11	Steere *et al.*, 1954
HYDROGONIUM		
consanguineum (Thw. *et* Mitt.) Hilp.	10	Gangulee and Chatterjee (present work)
SEMIBARBULA		
orientalis (Web.) Wijk. *et* Marg.	16	Banerji and Sen, 1957 (as *Barbula indica* Brid.); Gangulee and ohatterjee (present work)

With the data at present available, it is not possible to establish a clear relationship between the species and genera on a cytological basis but *Semibarbula orientalis* seems to be somewhat removed from the other species showing a higher chromosome number. Considering the morphology, Chen (1940) suggested that *Semibarbula* is a genus arising from *Barbula* and also showing some relationship with *Hydrogonium*. The reduced peristome of *Semibarbula* further supports such a view.

One heteromorphic pair of chromosomes has been noted in both *Hydrogonium consanguineum* and *Semibarbula orientalis*—which are dioecious. Nevertheless, it is premature to conclude that this pair controls the sex of the plants. It is necessary to examine the male and female gametophytes before arriving at a conclusion.

Both *Vesicularia montagnei* (Bel.) Fleisch. (Family *Hypnaceae*) and *Taxithelium nepalense* (Schwaegr.) Broth. (Family *Sematophyllaceae*) belong to the great group of Hypnoid mosses, sometimes included within a single family *Hypnaceae*. No cytological work seems to have been done on these two genera. There are 12 bivalent chromosomes in *Vesicularia montagnei* which can be sorted into three sizes. Other genera of *Hypnaceae* have shown basic chromosome numbers 6 to 12. No heteromorphic pair was noticed and this conforms to the fact that the species is known to be autoecious. *Taxithelium nepalense* showed 9 pairs of chromosomes of different sizes in the sporocytes. The only two other genera of *Sematophyllaceae* known to have been studied are *Brotherella* ($n = 10$) and *Heterophyllium* ($n = 11$). It is to be noted that in *Taxithelium nepalense* one pair of chromosomes separates precociously. Such precocious disjunction has been noticed by Steere *et al.* (1954) in *Fissidens limbatus* Sull. Steere *et al.* (1954) noted that the Californian race of *Weissia viridula* (L.) Hedw. shows 13 bivalents (1 very large, others equal) while the European race was previously found by Vaarama to contain 14 bivalents (all of equal size). This mutation is attributed to fragmentation of the large chromosome. A similar situation may be present in *Taxithelium nepalense*, where further investigation may show 9 as well as 10 chromosomed races.

Philonotis laxissima (C.M.) Bryol. java. showed 6 chromosomes in gametophytic squash. The genus seems to be very rigid in its chromosome number as all the 9 species examined by Yano and Vaarama as enumerated by Wylie (1957) as well as the present species show 6 chromosomes.

Summary

The haploid numbers (shown in brackets) were determined for the mosses *Hydrogonium consanguineum* (Thw. *et* Mitt.) Hilp. (10), *Semibarbula orientalis* (Web.) Wijk. *et* Marg. (16), *Vesicularia montagnei* (Bel.) Fleisch. (12), *Taxithelium nepalense* (Schwgr.) Broth. (9) and *Philonotis laxissima* (C.M.) Bryol. java. (6). One heteromorphic pair was noticed in each of *Hydrogonium consanguineum* and *Semibarbula orientalis*. Precocious separation of a bivalent was noticed in *Taxithelium nepalense*.

The cytotaxonomy of *Semibarbula orientalis* and the significance of the chromosome numbers in all the species are discussed.

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STUDIES IN INDIAN METZGERINEAE—IV*

Riccardia levieri Schffn.

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(Received for publication on July 11, 1959)

Riccardia Gray is represented in India by about ten species. Mitten (1860–61) was probably the first to record two species of *Riccardia* from India under the generic name *Sarcomitrium* Corda. Schiffner (1898 *a*) published a taxonomic account of 31 species of the genus from Java, Sumatra and Indo-Malaya, out of which 28 species were established by the author himself. The same year he (Schiffner, 1898 *b*) brought out a list of the species of the genus recorded till then from the East Indies, giving their synonyms and referring to the literature published on them. A year later Schiffner (1899) instituted *Riccardia levieri* the species dealt with in the present paper.

The first morphological study of *Riccardia*, as far as the author is aware, was pursued by Hoffmeister (1862) who described the apical cell and the sex-organs in *R. pinguis* (L.) Dum. and the capsule and gemmæ in *R. multifida* (L.) Dum. Subsequently the genus was investigated by Leitgeb (1877), who published the outlines of its morphology, based on his studies on *R. multifida*, *R. pinguis* and *R. alterniloba* Tayl. Clapp (1912) made a valuable contribution on the life-cycle of *R. pinguis*. Florin (1918) described abnormal archegonia in *R. pinguis*. Kashyap and Pandé (1922) worked out the morphology of *R. indica* (St.) Pandé *et* Srivastava. Showalter (1923 *a*) described some features of morphology of *R. pinguis*. In another paper he (Showalter, 1923 *b*) published an account of chromosomes in the same species, reporting ten as its haploid number. Steil (1923) described the antherozoids in three species of *Riccardia*, *i.e.*, *R. pinguis*, *R. palmata* (Hedw.) Dum. and *R. multifida*. Showalter (1925) described the germination of spore in *R. pinguis* and a year later he (Showalter, 1926 *a*) described the antherozoids in three different forms of *R. pinguis*. Simultaneously, Showalter (1926 *b*) published a critical account of the fertilization and early embryogeny in *R. pinguis*. Campbell (1928) gave a morphological account of *R. pinnatifida* Dum. Evans (1937) described the structure of the capsule wall in six species of *Riccardia*.

Material and Methods

Riccardia levieri is widely distributed in India. It occurs in Himalayas between an altitude of 6,000–8,000 ft. and in South India (Palni

* Part of this investigation was carried out by the author in the Botany Department, Lucknow University, Lucknow.

Hills, Bangalore, Canara). It is often found by the side of shady streams, on moist rocks and on fallen decaying logs and tree-trunks where it generally shows a very luxuriant growth. It has also been found growing epiphyllously on dicot leaves, collected from near Gersoppa Falls (Mysore State) in South India.

The material for this study was collected from dripping rocks by the side of a stream in Ranikhet (7,000 ft., Western Himalayas) and Kurseong (6,000 ft., Eastern Himalayas). The plants were killed in the field in Flemming's fluid (of various strengths), formalin alcohol, form-acetic alcohol, chromacetic acid with and without osmic acid, and Farmer's fluid. The material was dehydrated by passing through a close series of alcohols, cleared carefully in xylol-alcohol combinations. In some cases, butyl alcohol was substituted for xylol. It was embedded in paraffin in the orthodox way and sections were cut at the thickness of 4–10 microns. Safranin, gentian violet and Heidenhain's iron alum haematoxylin were used for staining.

Gametophyte

Schiffner (1899) described the details of the vegetative features and the young female shoot of *Riccardia levieri*. Subsequently the species was included by Stephani (1898–1900) and Kashyap (1929). Recently Pandé and Srivastava (1958) published an illustrated account of the taxonomy of this species.

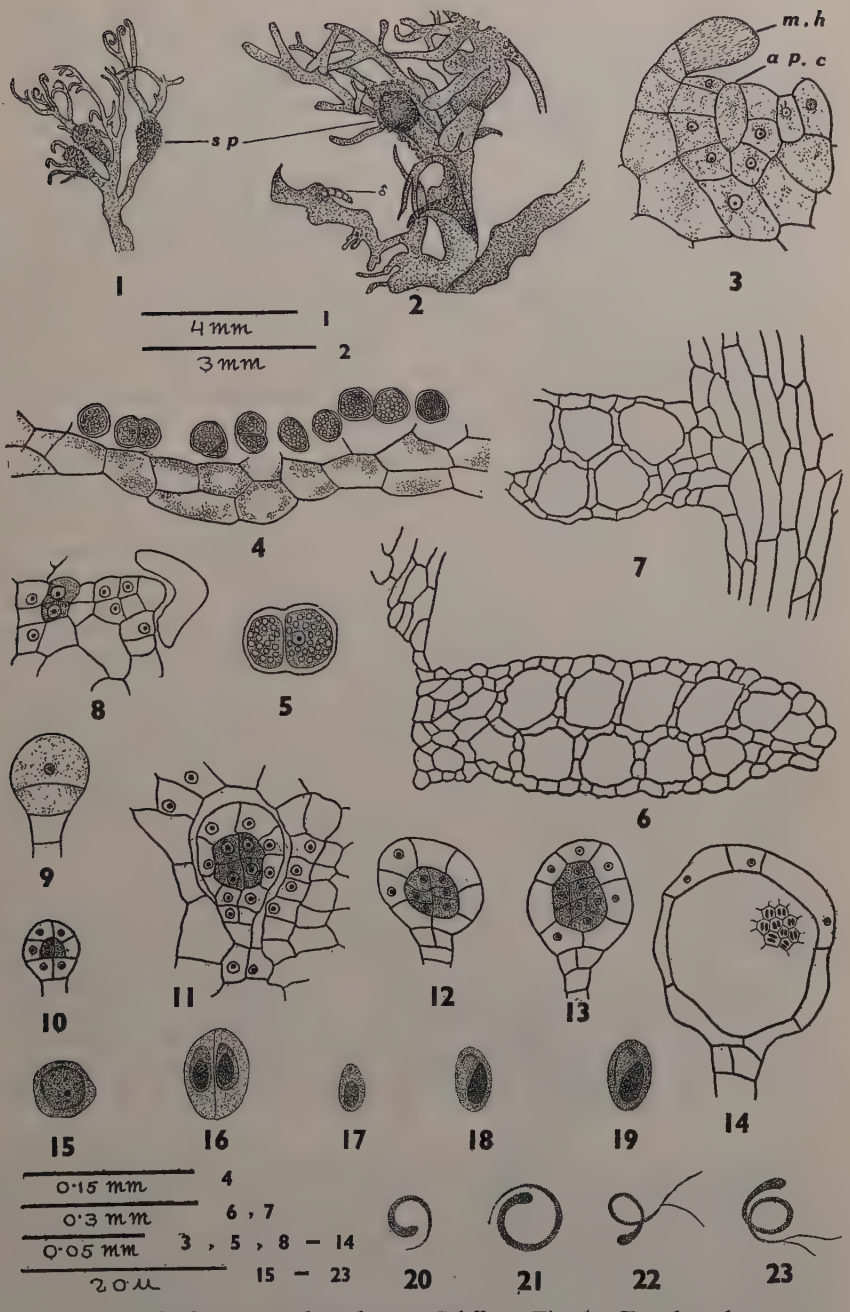
Riccardia levieri is not strictly dioecious (Text-Fig. 1, Plate XIV, Fig. 1) but both the antheridia and archegonia may sometimes occur on the same plant (Text-Fig. 2) as has already been noted by Pandé and Srivastava (1958).

The growing point and structure of the thallus.—The growing point of the plant lies at the apex in a notch and is covered over by caudueous mucilage hairs (Text-Fig. 3). The plant is profusely branched. Text-Figure 3 shows an apical cell in a horizontal section. It cuts off segments on its either side. Showalter (1923 *a*) has discussed in detail the apical cell and its segmentation in *Riccardia pinguis*.

The main shoot is about 8 cells thick in a cross-section. All the cells are parenchymatous and closely packed without any intercellular spaces. The epidermal cells are, however, somewhat smaller than the other cells of the thallus. There is no midrib but the thalli are always thicker along the centre and gradually thin out towards the lateral margins. The rhizoids are all smooth and present no peculiarities.

Bicellular gemmæ are frequently produced in *Riccardia levieri* (Text-Figs. 4, 5). They arise endogenously inside the epidermal cells and are more or less spherical (Text-Figs. 4, 5).

Sex organs.—The antheridia are borne on the upper surface (Text-Figs. 2, 7, 8) of the lateral branches. Each male shoot may bear 4 to 9



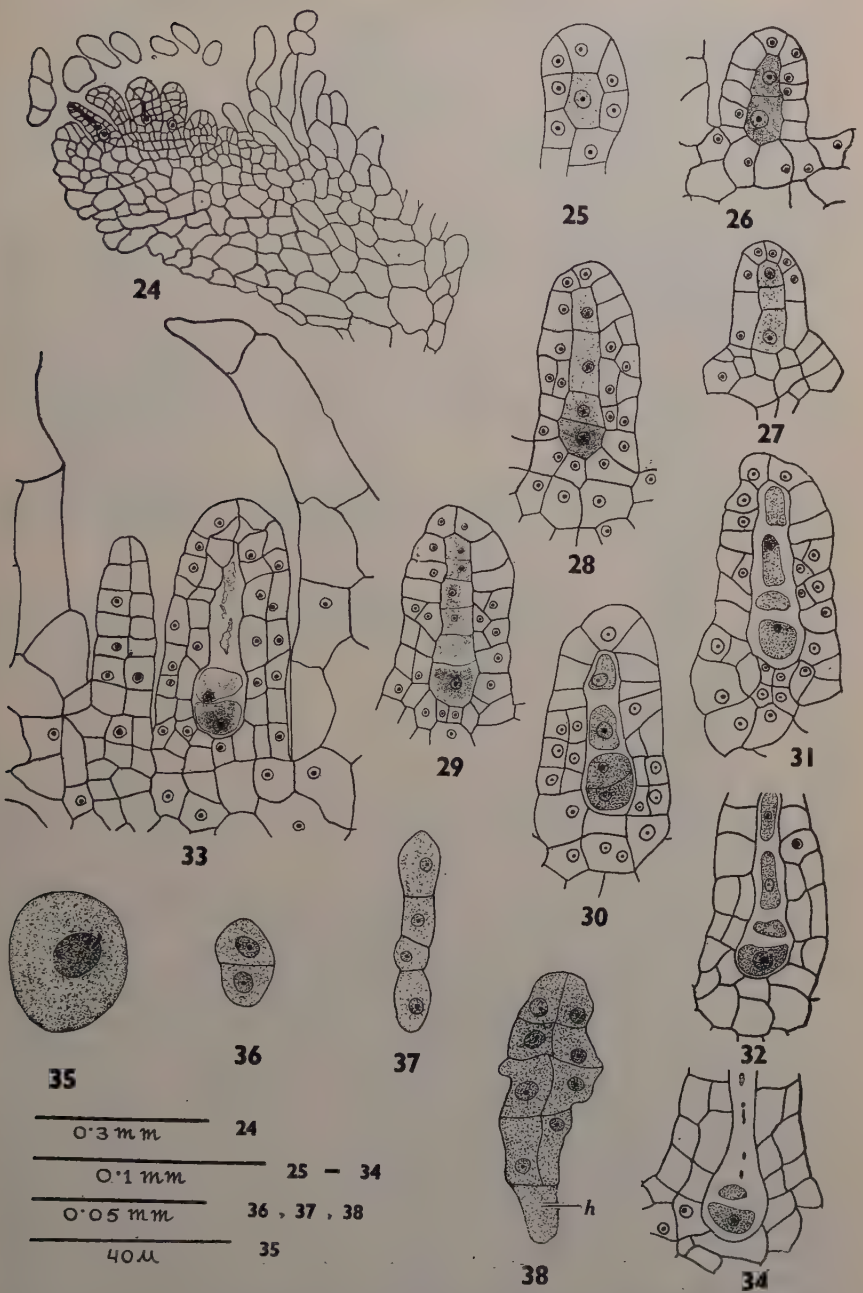
TEXT-FIGS. 1–23. *Riccardia levieri* Schffn. Fig. 1. Female plant; *sp.*, sporophyte. Fig. 2. A part of hermaphrodite plant; ♂, male shoot.

Fig. 3. Horizontal section apical cell; *ap.c.*, apical cells; *m.h.*, mucilage hair. Fig. 4. T.s. thallus showing gemmae. Fig. 5. Single gemma. Figs. 6–7. Horizontal section thallus showing male shoot. Figs. 8–13. L.s. antheridia in different stages of development. Fig. 14. L.s. mature antheridium. Fig. 15. A spermatid mother cell. Fig. 16. Sister spermatids separated by a thin membrane. Fig. 17. Spermatid showing blepharoplast. Figs. 18–19. Later stages of spermatids. Figs. 20–23. Spermatozoids.

pairs of antheridia. They are regularly produced in two alternating rows corresponding to the segments of the apical cell as described by Clapp (1912) for *Riccardia pinguis*. When first formed, the antheridia lie exposed on the surface of the thallus, but later they become embedded because of the rapid marginal growth of the thallus cells. The sequence of development, as far as followed, is the same as described for other species of *Riccardia* (Leitgeb, 1877 ; Clapp, 1912 ; Campbell, 1928). Some of the stages observed in the development of the antheridium are shown in Text-Figs. 9–14. The mature antheridium has a spherical body borne on a short multicellular stalk.

Spermatogenesis.—As the antheridium matures the spermatogenous cells divide repeatedly producing ultimately a large number of spermatid mother cells. The spermatid mother cells are rather small, but some of the details of the spermatogenesis could be studied. Text-Figure 15 shows a spermatid mother cell. It has a large and conspicuous nucleus and a small nucleolus, and is filled with fine granular hyaline cytoplasm. No trace of centrosome could be seen at this stage. The auhor's observations in this respect agree with those of Woodburn (1911, 1913) who did not find any centrosome-like structure up to the last division of the spermatid mother cell in liverworts. Text-Figure 16 shows two sister spermatids. These arise through a longitudinal division of the spermatid mother cell as in *Calycularia radiculosa* (Campbell, 1913) and not by a diagonal division as in *Marchantia polymorpha* (Ikeno, 1903). The spermatid has a prominent nucleus and also a nucleolus. The nucleus has great avidity for stains. The two spermatids are separated by a very thin membrane as has been stated by Campbell (1913) in the case of *Calycularia radiculosa*, and Campbell and Williams (1914) in *Pallavicinia zollingeri* and *P. levieri*. As the spermatid enlarges a dot-like blepharoplast is noticed at its narrower end (Text-Fig. 17). The origin of the blepharoplast could not be traced. The blepharoplast gradually increases in length and assumes more or less a cord-like appearance (Text-Figs. 18, 19). The nucleus of the spermatid later on elongates (Text-Figs. 20, 21) and is gradually transformed into a spermatozoid (Text-Figs. 22, 23). A mature spermatozoid has one or two coils with two flagellæ.

Archegonium.—*Riccardia levieri* bears archegonia on the dorsal surface of the lateral branches (Text-Fig. 24). Like the antheridia, the archegonia are also regularly arranged in two alternating rows. The archegonial shoots are very much smaller than the antheridial shoots. Each shoot may bear up to 20 archegonia. The sequence of development follows the usual course found in other members of the Metzgerineae. Some of the stages are shown in Text-Figs. 25–34. In mature archegonium



TEXT-FIGS. 24–38. *Riccardia levieri* Schffn. Fig. 24. V.l.s. female shoot. Figs. 25–33. L.s. archegonia in different stages of development. Fig. 34.

Lower part of mature archegonium. Fig. 35. Egg showing fertilization. Fig. 36. L.s. two-celled embryo. Fig. 37. L.s. four-celled embryo. Fig. 38. L.s. later stage of embryo; *h.*, haustorium.

the venter is two-layered, as in *R. pinguis* (Clapp, 1912 ; Showalter, 1923). The number of neck canal cells is about six. Both the ventral canal cell and the neck canal cells break down early in the same way, as reported by Clapp (1912) for *R. pinguis*.

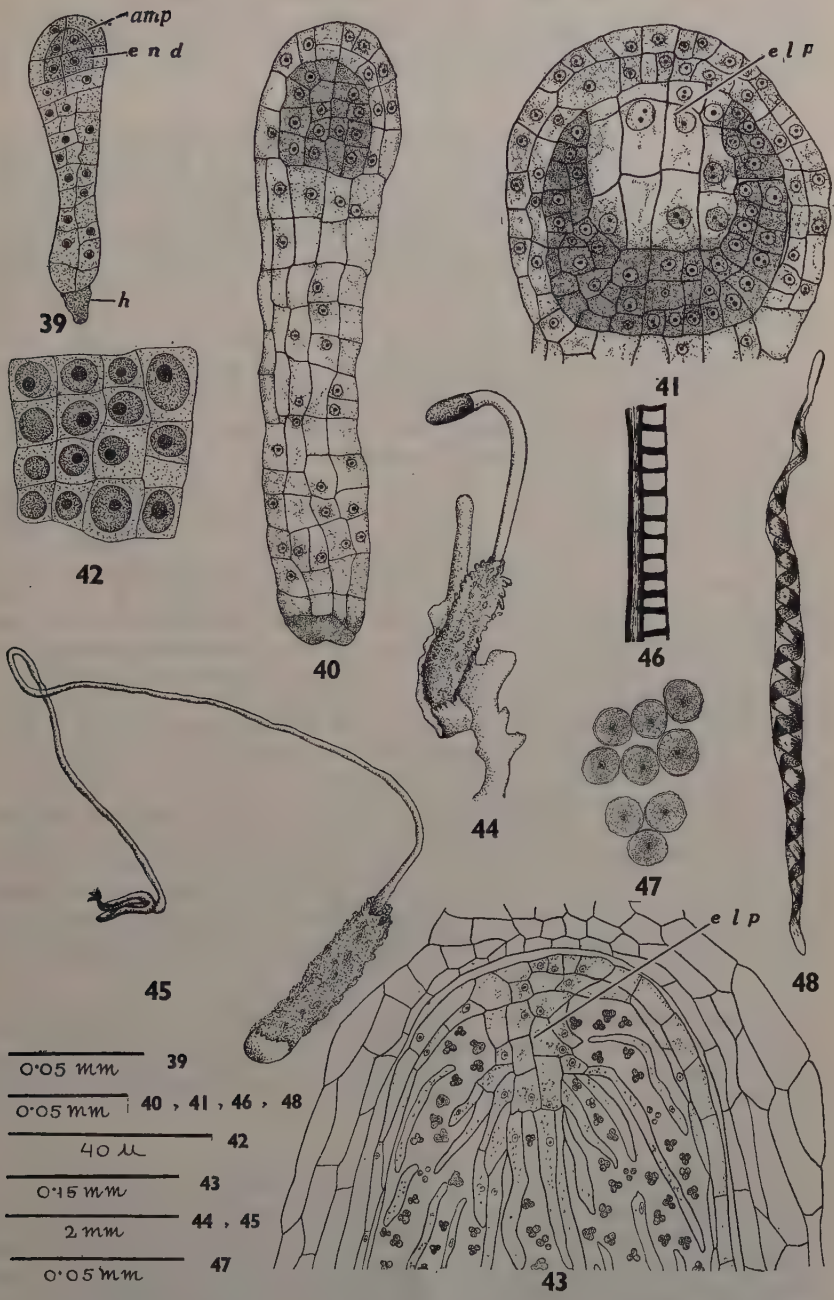
Fertilization.—Before fertilization both the neck and ventral canal cells of the archegonium are completely disorganised, forming a mucilaginous substance, which fills the entire neck and envelops the egg. The egg at this stage has a prominent nucleus which has great avidity for stains. In a few cases a number of sperm-like bodies were found embedded in the mucilage enveloping the egg, although the actual act of fertilization was not seen. Text-Figure 35, however, shows a mature egg with a sperm-like body penetrating the egg nucleus. The egg has a definite membrane.

Soon after fertilization the cells of the venter and the thallus below the archegonium divide and form a thick calyptra which protects the young embryo. The calyptra carries on its body the remains of unfertilized archegonia.

Coker (1909) has reported two embryos inside one calyptra. A somewhat similar condition has been observed by the author in *Riccardia levieri* (Plate XIV, Figs. 3, 4).

Sporophyte

In *Riccardia levieri*, as in *R. pinguis* (Clapp, 1912; Showalter, 1923) the first division in the fertilized egg is transverse (Text-Fig. 36) dividing it into an epibasal cell, which develops the main embryo, and a hypobasal cell which forms the haustorium. In *R. pinguis* (Clapp, 1912) a transverse wall is laid down in the epibasal cell forming a three-celled embryo. This is followed by another transverse division in the uppermost cell producing a four-celled filamentous embryo. According to Showalter (1923 *a*) the first division in the epibasal cell (in the two-celled embryo) is parallel to the long axis of the archegonium, the upper two cells form the embryo proper while the lower cell produces the haustorium. A study of Text-Figs. 36 and 37 of this paper would suggest that in *R. levieri* also a four-celled filamentous embryo is produced as has been observed by Clapp (1912) in *R. pinguis*. The sequence of segmentation, however, could not be followed. The next stage observed is shown in Text-Fig. 38. The embryo proper consists of four rows of quadrants. The upper two of these presumably produce the capsule, the middle forms the seta, while the lowest one, excluding the haustorium, forms the foot. A later stage in embryogeny is shown in Text-Fig. 39. The various regions of the sporophyte have been delimited. In the region of the capsule the amphithecium and endothecium have been separated through periclinal walls. The endothecium, *i.e.*, the central



TEXT-FIGS. 39–48. *Riccardia levieri* Schffn. Fig. 39. L.s. young sporophyte showing differentiation into capsule, seta and foot; *end.*, endothecium; *amp.*,

amphithecium; *h.*, haustorium. Fig. 40. L.s. later stage of developing sporophyte. Fig. 41. L.s. young capsule; *elp.*, elaterophore. Fig. 42. Archesporial cells. Fig. 43. L.s. apical part of capsule showing young elaters attached to the elaterophore (*elp.*). Figs. 44–45. Sporophytes. Fig. 46. Capsule wall. Fig. 47. Spore. Fig. 48. Elater.

tissue consists of eight cells (only four are seen in longitudinal section), four in each tier. In this respect *R. levieri* resembles *R. pinguis* (Clapp, 1912) but further details of the segmentation of the endothecial cells leading to the differentiation of the apical cap could not be followed. In *R. pinguis* (Clapp, 1912, p. 184), out of the eight endothecial cells, "the lower four divide by horizontal and vertical walls ; the upper also divide, but form only a group of sterile cells—a cap, later continuous with the elaterophore".

Text-Figure 40 shows an older embryo. The archesporial cells show denser contents and prominent nuclei. Some of the amphithecial cells have divided by periclinal walls. The process of cell division in these cells is not simultaneous, hence the capsule wall at places is yet only one-layered (Text-Fig. 40). Ultimately the entire wall becomes two-layered (Text-Fig. 41). The cells in the different regions of the capsule show difference in their rate of growth and contents. The cells of the lower and peripheral region have denser contents while those of the central region are poorer in contents (Text-Fig. 41). The divisions in the latter are not so rapid as in the former and hence they become somewhat elongated. These cells ultimately produce the elaterophore (Text-Fig. 43). The cells of the endothecium surrounding the elaterophore divide actively and form a large number of archesporial cells which have dense contents and large nuclei (Text-Fig. 42). Ultimately these differentiate into spindle-shaped elongated cells which form the elaters and the cubical cells which produce the spores. Text-Figure 43 shows the upper part of a young capsule in longitudinal section. Some young elaters are seen attached to the elaterophore. The fully formed elaterophore is a long cylindrical structure extending to about one-third in the cavity of the capsule. A number of elaters are always attached to it in a spreading manner. These elaters are rather short and stumpy and each one of the four pieces into which the elaterophore breaks up bears a few of these elaters (Plate XIV, Fig. 2).

Calyptra.—In *Riccardia levieri*, like other species of the genus, the young sporophyte is protected by a thick club-shaped calyptra. As there is complete absence of other protective layers, such as perianth and involucre, the calyptra is very massive (Text-Figs. 44 and 45). It has warts all over its surface.

Foot.—The foot in *Riccardia levieri* is cylindrical and its end is more or less rounded and is not very much marked off from the seta. It remains anchored within the tissue of the gametophyte. Due to the pressure of the foot, the cells of the gametophyte surrounding the foot are crushed and form a mucilaginous substance which is presumably utilised by the developing sporophyte.

Seta.—The seta in a young sporophyte is very short and thick, and its transverse walls are longer than the radial walls. When the spores mature, the seta elongates very quickly and becomes long and thin (Text-Fig. 45), its cells becoming many times longer than broad.

Capsule.—The capsule is cylindrical, and dark brown. It remains enclosed within the club-shaped calyptra till the spores are mature. The elaterophore hangs down in the cavity of the capsule. A few elaters are always found attached to the elaterophore. The capsule when ripe, dehisces by four valves separating completely from the apex to base. Each one of the four valves carries a piece of elaterophore and a few fixed elaters. The wall of the capsule is two-layered with thickenings on the outer layer of its cells (Text-Fig. 46). The spores (Text-Fig. 47) are finely granulose and about 14 microns in diameter. The elater (Text-Fig. 48) is spindle-shaped with one brown thickening on its wall, as in other species of the genus.

Summary

1. *Riccardia levieri* Schffn. occurs commonly in the Himalayas (6,000–8,000 ft.).

2. The plant forms thick caespitose patches.

3. The apical cell lies at the apex of the branches and cuts off two lateral sets of segments.

4. The antheridia are formed on the upper surface of the lateral branches. The development of the antheridium is as in other species of the genus.

5. Some stages of spermatogenesis have been described. The sister spermatids are separated by a definite membrane.

6. In spermatids in early stages the blepharoplast is dot-like but later on it elongates and becomes cord-like.

7. The archegonia are borne on the dorsal surface of lateral shoots. The sequence of development follows the usual Metzgerineae type.

8. A thick calyptra is formed after fertilization which protects the developing sporophyte. The calyptra is rough and warty and carries on its surface the remains of the unfertilized archegonia.

9. Abnormally two sporophytes may be formed inside a common calyptra.

10. The first division in the fertilized egg is transverse to the long axis of the archegonium. Subsequently two transverse walls are laid down in the epibasal cell which forms the embryo proper. The hypobasal cell forms the haustorium. The embryo is of filamentous type.

11. The elaterophore is well developed and extends to about one-third the length of the capsule. It is differentiated within the endothecium.

12. The wall of the capsule is two-layered with pronounced annular thickenings on the wall of the outer layer.

13. The capsule dehisces by four valves extending nearly to the base of the capsule. Each of the valves carries attached to it, a quadrant of the elaterophore and a few fixed elaters.

14. The spores are finely granulose and the elater has a single spiral band of thickenings on its wall.

Acknowledgement

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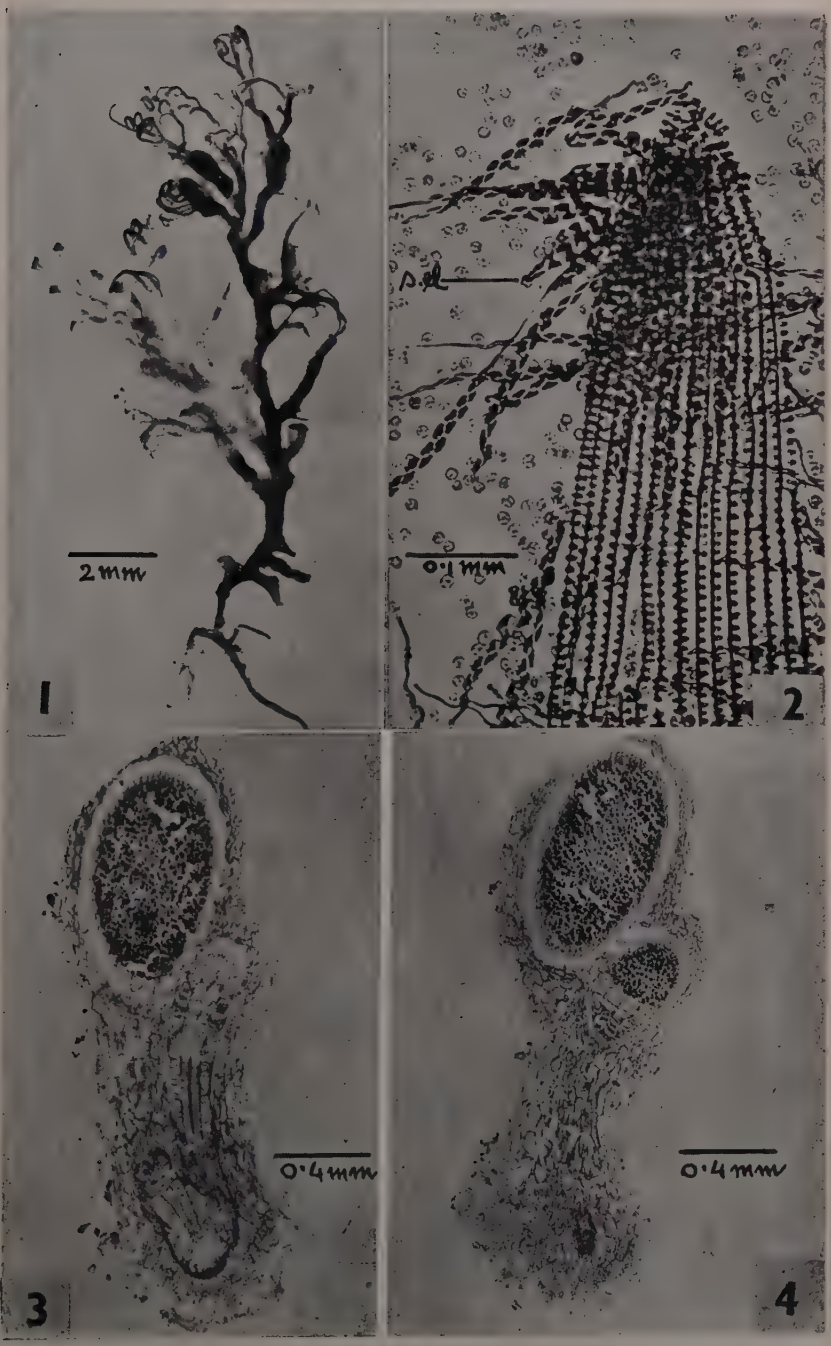

K. P. Srivastava

FIGS. 1–4

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* Not seen in original.

EXPLANATION OF PLATE XIV

FIG. 1. A female plant.

FIG. 2. A valve from dehisced capsule; *s.el.*, Stumpy elater.

FIGS. 3 & 4. L.s. two young sporophytes inside one calyptra.

PRELIMINARY OBSERVATIONS ON THE FLORA OF DILAPIDATED WALLS AND BUILDINGS OF CALCUTTA AND ITS SUBURBS

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Although a considerable amount of work has been done on forest ecology, vegetational types, autecology and synecology in India during recent years, yet our knowledge of the flora, which occurs on dilapidated walls and buildings, is very meagre.

Location and Topography

The city of Calcutta lies between 88½° longitude and 22° 34′ latitude in West Bengal. It is situated on the bank of Hooghly River. The suburbs of Calcutta, such as Dum Dum, Sinthi, Bagmari and Dakshineswar are little cut off from the town itself. The investigated areas in Calcutta may broadly be divided into 3 zones—South, Central and North respectively.

Climate

Calcutta possesses a monsoon type of climate with a dry cool winter from mid October to the end of February; and a dry hot summer from March to early June. The duration of rainy season is from May to October or normally the rainy season starts from middle of June and ends by the end of October. The rainfall is, as a rule, very irregular and uneven ; it falls either in torrents or in evenly spread showers; thus it so happens that all the localities do not get properly and equally drenched.

For the period under investigation the total rainfall is 116·53 cm. per annum, the heaviest rainfall is 34·29 cm. being recorded between July and August 1958. The mean maximum temperature in April 1958 and June 1958 is about 36·5°C. and mean minimum temperature in January 1958 is 15·8° C. The maximum humidity is 87% in May 1958 and 87–90% in July and August 1958 and the minimum humidity of the same months is 49% and 67–69% respectively.

It has been further observed that while summer temperatures show a gradual rise, the rel..tive humidity falls accordingly; this causes a slow desiccation in the areas under study.

TABLE I

Meteorological data from November 1957 to March 1959

Month and Year	Temperature in °C.		% Relative humidity		Total rainfall in cm.
	Mean maximum	Mean minimum	Mean maximum	Mean minimum	
November 1957	36·6	17·5	95	37	0·0
December 1957	28·2	14·6	94	37	0·0
	32·4	16·05	94·5	37	0·0
January 1958	28·9	15·8	97	36	1·00
February 1958	30·0	17·4	93	34	7·11
March 1958	35·8	21·2	88	22	0·50
April 1958	37·0	25·6	87	38	3·30
May 1958	36·0	27·2	87	49	5·84
June 1958	36·7	27·6	89	53	9·90
July 1958	32·4	26·4	90	69	34·29
August 1958	32·3	26·5	90	67	18·03
September 1958	32·6	26·1	90	66	27·17
October 1958	32·5	24·9	91	58	7·11
November 1958	30·2	20·3	93	49	2·28
December 1958	27·8	15·7	96	44	0·00
	32·6	22·8	90·9	48·7	116·53
January 1959	26·9	15·1	94	44	2·03
February 1959	28·7	16·2	88	33	1·77
March 1959	34·5	22·3	90	29	0·76
	30·3	17·8	90·6	35·3	4·56

MATERIAL AND METHODS

Altogether eighteen walls with as many diverse features as can be possibly considered have been selected in Calcutta proper and some of

its suburbs: Sinthi, Bagmari, Dum Dum and Dakshineswar. With respect to their exposure to the sun the walls are oriented as follows: 5, eastern; 4, western; 4, northern; 2, southern; 2, north-eastern; and 1, south-eastern.

Observations have been made at 7-day intervals and the constituent flora on each wall with their periodic incidence and morphological manifestations are recorded and determined.

Observations

Vegetation

The edaphic and biotic factors show a significant bearing on the local wall-flora as summarised below.

(*a*) *Edaphic.*—The edaphic factor which appears to influence the wall-flora of old houses is mainly the nature of the exposed surfaces, which are generally characterised by cracks and crevices. The latter contains disintegrated bricks and mortar, decayed remnants of plant parts and also debris.

The ultimate source of water to the exposed surfaces is periodical rainfall, and also water trickling intermittently from the leaking water-tanks; and other sources situated above. The amount of water held in the adhering soil on the wall, from the sources enumerated above, appears to be directly governed by the nature of the substratum.

(*b*) *Biotic.*—Among the agents affecting normal and unhindered development of vegetation in this area are chiefly the birds: crows, sparrows, bats, etc., and to a considerable extent rodents, cats and man; all together play not an insignificant role in shaping the vegetational set-up. Yet another source of disturbance is provided by hordes of insects.

It has been further observed that some plants growing on dilapidated walls are attacked by pathogenic fungi and some also harboured by termites; these drastically retard the growth of the particular species concerned in the association.

Changes in construction or repairs to houses—old and new—interrupt the development of wall flora.

The constituent elements of the flora include 81 species spread over 76 genera and 36 families of angiosperms.

Altogether 18 wall sites have been examined. Those of the suburbs of Calcutta appear to be the richest in flora, having 51–79 species out of a total of 81 species recorded for the whole town. Of these 8 species are exclusive to the area, *viz.*, *Argemone mexicana*, *Rorippa indica*, *Abutilon indicum*, *Urena lobata*, *Triumfetta bartramia*, *Sonchus asper*, *Andrographis paniculata*, *Peristrophe bicalyculata*. In fact, these are common species of the ground and have taken to the walls in the suburbs as a result of competition at lower levels. The roads in the suburbs

List of plants observed on dilapidated walls and buildings of Calcutta proper and some of its suburbs

Sl. No.	Names of Plants	Sl. No.	Name of Plants
1	*Argemone mexicana* L.	41	*Nicotiana plumbaginifolia* Viv.
2	*Rorippa Indica* (L.) Hochreut	42	*Lindenbergia indica* (L.) O. Ktze
3	*Polanisia viscosa* L.	43	*Lindernia crustacea* (L.) Muell
4	*Portulaca quadrifida* L.	44	*Scoparia dulcis* L.
5	*Abutilon indicum* Don.	45	*Dipteracanthus prostratus* (Poir)
6	*Urena labata* L.	46	*Hemigraphis hirta* T.
7	*Triumfetta bartramia* Linn.	47	*Andrographis paniculata* Nees
8	*Corchorus capsularis* L.	48	*Rungia parviflora* Nees
9	*Oxalis corniculata* L.	49	*Peristrophe bicalyculata* Nees
10	*Azadirachta indica* Juss.	50	*Justicia diffusa* Willd.
11	*Cedrela toona* Roxb.	51	*J. simplex* Don.
12	*Zizyphus mauratiana* Lamk.	52	*Lantana camara* L.
13	*Cayratia carnosa* Gagnep.	53	*Phyla nodiflora* (L.) Gr.
14	*Mangifera indica* L.	54	*Ocimum sanctum* L.
15	*Desmodium gengeticum* Dc.	55	*Leucas procumbens* Desf.
16	*Cassia sophera* L.	56	*Boerhaavia repens* L.
17	*Tamarindus indica* L.	57	*Amarantus spinsosus* L.
18	*Syzygium cumini* (L.) SK.	58	*A. viridis* L.
19	*Carica papaya* L.	59	*Achyranthes aspera* L.
20	*Coriandrum sativum* L.	60	*Alternanthera sessiles* Br.
21	*Dentella repens* Forst.	61	*Peperomia pellucida* Kunth
22	*Oldenlandia corymbosa* L.	62	*Euphorbia hirta* L.
23	*O. paniculata* L.	63	*Phyllanthus niruri* L.
24	*Vernonia cinerea* Less.	64	*Croton sparciflorus* Morung.
25	*Ageratum conyzoides* L.	65	*Chrozophora plicata* A. Juss
26	*Eupatorium odoratum* L.	66	*Acalypha indica* L.
27	*Blumea lacera* Dc.	67	*Tragia involucrata* L.
28	*Eclipta prostrata* (Linn.)	68	*Trema orientalis* Bl.
29	*Synedrella nodiflora* Gaertn.	69	*Ficus bengalensis* L.
30	*Tridax procumbens* L.	70	*F. religiosa* L.
31	*Sonchus asper* Vill.	71	*F. hispida* L.
32	*Lochnera rosea* (L.) Reichb.	72	*Fleurya interrupta* Gaud
33	*Calotropis procera* Br.	73	*Pilea microphylla* Liebm.
34	*Canscora diffusa* Br.	74	*Pouzolzia indica* Gaud
35	*Heliotropium indicum* L.	75	*Commelina bengalensis* L.
36	*Ipomea quamoclit* L.	76	*Typhonium trilobatum* Schoot
37	*Solanum nigrum* L.	77	*Kyllingia brevifolia* Rottb.
38	*Lycopersicum esculentum* Mill.	78	*Cyperus rotundus* L.
39	*Capsicum frutescens* L.	79	*Panicum flavescens* Sw.
40	*Datura metel* Linn.	80	*Cynodon dactylon* Pers.
		81	*Eragrostis pilosa* Beauv.

are generally dusty and walls are not so much cared for; and thus the species, having escaped from competition lower down, thrive well on the wall substratum.

There are about 35 species which can be said to be truly characteristic of the wall-flora with a frequency distribution of more than 70%. The lesser frequent species appear to be adventives. The wall species are characterised by possession of (*a*) sticky and small seeds, (*b*) perennating rhizomes and root stocks, (*c*) high reproductive capacity and (*d*) high lime requirement. Species of *Ficus* particularly *Ficus religiosa* shows the highest frequency. Other two tree species showing high frequency are *Azadirachta indica* and *Mangifera indica* and also the shrub *Lantana*. However, these can at the best be regarded as accidentals to the wall flora. The seeds get lodged in crevices and germinate, but the plants do not proceed growing beyond the stage of sapling.

The most successful herbaceous species are:—

Cyperus rotundus, *Lindenbergia indica*, *Scoparia dulcis* and species of *Ammania*, *Blumea*, *Eclipta*, *Tridax*, *Justicia*, *Oldenlandia*, etc.

As compared with terrestrial plants the wall flora show, in general, stunted appearance and are, in general, xeromorphic in character. The root-systems are in most cases superficial in nature with spreading branches, the ends penetrating the crevices of the substratum. Plants like *Azadirachta indica*, *Cedrela toona*, *Ficus hispida*, *F. religiosa*, *F. bengalensis*, *Lantana camara*, *Syzygium cumini*, *Trema orientalis*, *Cassia sophera*, *Mangifera indica*, *Eupatorium odoratum*, *Zizyphus mauratiana*, *Calotropis procera*, show the presence of deeply penetrating tap-roots with few lateral branches which extend laterally in the substratum.

Conspicuous variations of morphological nature have not been observed in the various species constituting the wall flora. However, there are definite variations in the (*a*) height of plants and (*b*) the size, shape and nature of their leaves.

The reduction in height has been specially noted in *Lindenbergia indica*, *Justicia simplex*, *Vernonia cinerea*, *Euphorbia hirta*, *Acalypha indica*, *Croton sparciflorus*, *Lindernia crustacea*, *Polanisia viscosa*, *Portulaca quadrifida*.

Reduction in size of leaves has been noted in such plants, as *Croton sparciflorus*, *Peristrophe bicalyculata*, *Ageratum conyzoides*, *Lindernia crustacea*, *Canscora diffusa*, *Pouzolzia indica*, *Capsicum frutescens*. Succulence of leaves has also been observed in *Phyla nodiflora*, *Portulaca quadrifida*, *Oldenlandia paniculata*, *Peperomia pellucida*, *Calotropis procera*.

Seasonal Succession of Plants

During the hottest part of the year between April and May 1958 when the temperature reaches 40–43° C. almost all the vegetation on

the exposed walls die down completely excepting the woody and shrubby plants which perennate in an almost leafless condition.

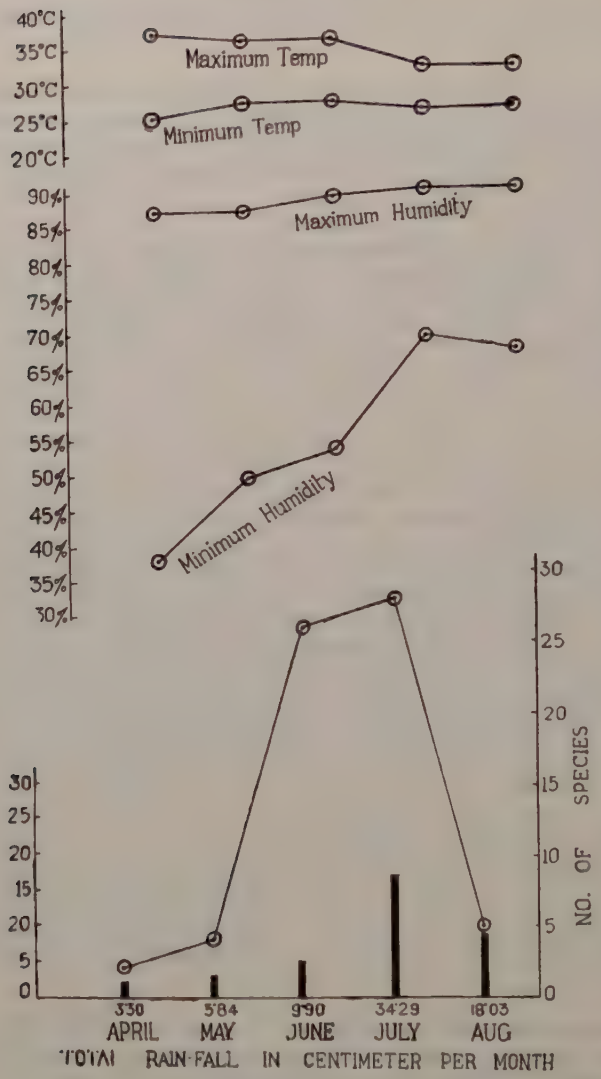
Soon after the first outbreak of the monsoon towards the end of April 1958, seedlings of *Pilea microphylla*, *Peperomia pellucida* appeared almost simultaneously on the walls. These are followed by *Boerhaavia repens*, *Tridax procumbens*, *Panicum flavescens*, *Oldenlandia corymbosa* in the month of May 1958.

With the onset of monsoon in the month of June 1958, the plants such as, *Lindenbergia indica*, *Scoparia dulcis*, *Euphorbia hirta*, *Eclipta prostrata*, *Lindernia crustacea*, *Achyranthes aspera*, *Dentella repens*, *Alternanthera sessilis*, *Oxalis corniculata*, *Dipteracanthus prostratus*, *Amarantus spinosus*, *Leucas procumbens*, *Phyllanthus niruri*, *Croton sparciflorus*, *Cynodon dactylon*, *Urena lobata*, *Synedrella nodiflora*, *Cyperus rotundus*, *Justicia diffusa*, *Nicotiana plumbaginifolia*, *Rorippa indica*, *Capsicum frutescens*, *Eragrostis pilosa*, *Triumfetta bartramia*, *Oldenlandia paniculata*, *Polanisia viscosa*, appear on the walls simultaneously and they are followed by *Vernonia cinerea*, *Ageratum conyzoides*, *Eupatorium odoratum*, *Portulaca quadrifida*, *Blumea lacera*, *Phyla nodiflora*, *Kyllingia brevifolia*, *Justicia simplex*, *Fleurya interrupta*, *Rungia parviflora*, *Acalypha indica*, *Ocimum sanctum*, *Heliotropium indicum*, *Commelina bengalensis*, *Argemone mexicana*, *Canscora diffusa*, *Andrographis paniculata*, *Lochnera rosea*, *Coriandrum sativum*, *Amarantus viridis*, *Cayratia carnosa*, *Corchorus capsularis*, *Lycopersicum esculentum*, *Ipomea quamoclit*, *Sonchus asper*, *Tragia involucrata*, *Chrozophora plicata*, *Solanum nigrum* in the month of July 1958 or so when definite monsoon conditions prevail. These plants are followed by five species in the month of August 1958, namely, *Abutilon indicum*, *Carica papaya*, *Desmodium gangeticum*, *Pouzolzia indica*, and *Hemigraphis hirta*.

The woody and shrubby perennials, such as, *Azadirachta indica*, *Cedrela toona*, *Calotropis procera*, *Cassia sophera*, *Lantana camara*, *Ficus hispida*, *F. religiosa*, *F. bengalensis*, *Mangifera indica*, *Syzygium cumini*, *Tamarindus indica*, *Trema orientalis*, *Zizyphus mauratiana*, commence their vegetative activities once again in the months of July and August 1958.

It is apparent that the majority of species are herbaceous. They are either annuals, such as, *Lindenbergia indica*, *Hemigraphis hirta*, *Justicia simplex*, *Rungia parviflora*, *Pouzolzia indica*, etc., or perennials, such as, *Phyla nodiflora*, *Boerhaavia repens*, *Portulaca quadrifida*, etc.; 23·4% approximately represent small and woody shrubs, such as, *Andrographis paniculata*, *Eupatoriun odoratum*, *Abutilon indicum*, *Zizyphus mauratiana*, *Cassia sophera*, etc., and 2·4% approximately climbers, such as, *Ipomea quamoclit*, *Cayratia carnosa*.

The accompanying graph (Text-Fig. 1) represents the number of species between April and August as also the relative humidity and the maximum and minimum temperatures prevailing during the period.



TEXT-FIG. 1

It will be noted that from May to July there is a sharp rise in the number of species which abruptly comes down in August with the cessation of the monsoon showers.

Longevity of the Plants

It has been found that about 2·4% of the species live for 5 months, as for example, *Fleurya interrupta*, *Desmodium gangeticum* and an equal

number for six months, such as, *Lindenbergia indica*, *Lycopersicum esculentum* ; 13·5% species live for 7 months, such as, *Canscora diffusa*, *Corchorus capsularis*, *Pouzolzia indica*, *Solanum nigrum*, *Carica papaya*, *Lindernia crustacea*, *Phyllanthus niruri*, *Rorippa indica*, *Oldenlandia paniculata*, *Polanisia viscosa*, *Ageratum conyzoides*; 23·4% for 8 months, such as, *Acalypha indica*, *Andrographis paniculata*, *Lochnera rosea*, *Cayratia carnosa*, *Typhonium trilobatum*, *Peristophe bicalyculata*, *Tragia involucrata*; 18·5% for 9 months as typified by *Peperomia pellucida*, *Scoparia dulcis*, *Euphorbia hirta*, *Dentella repens*, *Dipteracanthus prostratus*, *Synedrella nodiflora*, *Blumea lacera*, *Rungia parviflora*, *Ocimum sanctum*, *Amarantus viridis*, *Sonchus asper*; 17·2% for 10 months, such as, *Argemone mexicana*, *Capsicum frutescens*, *Justicia diffusa*, *Urena lobata*, *Leucas procumbens*, *Amarantus spinosus*, *Alternanthera sessiles*, *Oxalis corniculata*, *Oldenlandia corymbosa*; 3·7% for 11 months, as for example, *Boerhaavia repens*, *Tridax procumbens*, *Panicum flavescens* and 20% are perennials; the latter include such herbaceous plants as, *Protulaca quadrifida*, *Phyla nodiflora*; woody and shrubby plants as, *Ficus hispida*, *F. bengalensis*, *F. religiosa*, *Cedrela toona*, *Azadirachta indica*, *Calotropis procera*, *Mangifera indica*, *Lantana camara*, *Syzygium cumini*, *Tamarindus indica*, *Cassia sophera*, *Trema orientalis and Zizyphus mauratiana*.

It may be mentioned here that the period of longevity of plants depends on the topography of the walls, direction of the sun and wind and source of water-supply in the case of annuals. Nevertheless, the variations are not very significant.

Dormancy of Seeds

The period of dormancy of seeds of plants growing on dilapidated walls and buildings so far studied is not very remarkable. It varies from one to five months. It was noted that 9·8% of seeds are dormant for 2 months, such as, *Justicia simplex*, *Rungia parviflora*, *Oxalis corniculata*, *Croton sparciflorus*, etc.; 30·8% for three months, such as, *Scoparia dulcis*, *Euphorbia hirta*, *Eclipta prostrata*, *Dentella repens*, *Alternanthera sessiles*, *Amarantus viridis*, *Hemigraphis hirta*, *Boerhaavia repens*, *Corchorus capsularis*, etc.; 25·9% for four months as exemplified by *Ageratum conyzoides*, *Achyranthes aspera*, *Canscora diffusa*, *Triumfetta bartramia*, *Phyllanthus niruri*, *Dipteracanthus prostratus*, *Datura metel*, *Coriandrum sativum*, *Peristrophe bicalyculata*, *Cayratia carnosa*, *Cynodon dactylon*, *Panicum flavescens*, etc. ; 14·8% for five months, such as, *Nicotiana plumbaginifolia*, *Rorippa indica*, *Abutilon indicum*, *Tragia involucrata*, *Pouzolzia indica*, etc. ; 1·2% for six months, such as, *Pilea microphylla* and 16·04% are perennials, such as, *Azadirachta indica*, *Cedrela toona*, *Zizyphus mauratiana*, *Tamarindus indica*, *Cassia sophera*, *Trema orientalis*, etc.

The shortest period of dormancy is illustrated by *Tridax procumbens* where it is only 45 days and the longest period by *Pilea microphylla* where it is approximately six months.

Dispersal of Seeds

The chief agents for dispersal of seeds of wall flora are birds, wind, human beings and insects. The seeds of *Corchorus capsularis*, *Carica papaya*, *Euphorbia hirta*, *Acalypha indica*, *Ficus hispida*, *F. bengalensis*, *F. religiosa*, *Syzygium cumini*, etc., are dispersed by birds ; those of *Vernonia cinerea*, *Calotropis procera*, *Tridax procumbens*, *Sonchus asper*, *Canscora diffusa*, etc., by the agency of wind, and those dispersed by human agency and other animals are represented by *Achyranthes aspera*, *Mangifera indica*, *Tamarindus indica*, *Zizyphus mauratiana*, etc. ; sometimes insects are responsible for the removal of seeds from one place to another, such cases are typified by *Coriandrum sativum*, *Phyllanthus niruri*, *Heliotropium indicum*, etc.

Conclusions

Preliminary study of wall flora of Calcutta and its suburbs showed that a large number of phanerogams widely different in their systematic positions colonize the cracks and crevices of the walls. Observations conducted during the two consecutive seasons in 1957–59 showed mostly the same type of vegetation appearing at the same place year after year. This leads one to infer that these plants are derived from the seeds of the plants of the previous generations which had remained dormant and sprouted as soon as favourable conditions prevailed. The occurrence of a fresh element at a particular locality could be attributed to the agency of birds and beasts or insects.

The importance and bearing of water to the development of vegetation of dilapidated walls and buildings appeared to be very great. Luxuriant vegetation was associated with constant trickling of water. During the monsoon all the plants situated on dilapidated walls and buildings grew profusely due to abundance water-supply and also on account of higher humidity of the atmosphere. As a matter of fact, water was the determining factor in the life of the wall flora. The wall flora, generally annuals, showed signs of death and decay at the end of the monsoon.

Temperature also appeared to be equally important. It was observed that the plants occurring in shady situation persisted for a longer period provided constant water supply was ensured.

Other factors in determining the growth and longevity of the wall flora were the exposure to the sun and topography of the substratum; both of these appeared of little significance.

Summary

The study of the wall flora of a few selected localities in Calcutta and its suburbs showed the occurrence of 81 species distributed in 76 genera and 36 families of angiosperms. These included mostly annuals and perennials; the former appeared at about the same time during two successive years indicating thereby that the seeds remained dormant

in situ, till favourable conditions prevailed. The perennials remained mostly in an aphyllous condition during the summer and recommenced growth and activity with the commencement of the monsoon.

Temperature, topography of the wall and its exposure to the sun were also of importance in determining the types of vegetation and the longevity of plants.

Acknowledgment

I desire to express my sincere gratitude to Dr. I. Banerji, Head of the Department of Botany, Calcutta University, for suggesting this problem and his keen interest in the work and to Dr. P. Kachroo, Chief Botanist, Malaria Research Laboratory, D.V.C., Burdwan, for valuable suggestions. My thanks are also due to the Director, Alipore Meteorological Office, Calcutta, for supplying me meteorological data and to the Ministry of Education, Government of India, for the award of a Senior Scientific Research Training Scholarship.

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ON THE DIATOM FLORA OF SOME PONDS AROUND VASNA VILLAGE NEAR AHMEDABAD

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It is for the first time that the Diatom flora of temporary ponds of India and particularly of Ahmedabad is studied. These ponds owe their origin from large depressions or through unplanned excavations of land for road construction or otherwise. During the rains these get filled in to varying degrees and become the source of water-supply for various purposes of men and beasts alike. The accumulated water thus was found to be constantly disturbed and polluted. Depending upon the size of these low grounds, the area being anywhere between 75 – 400 sq. meters, the water was found to last in them for varying periods of time after the last showers of the wet period were over.

The ponds, 5 in number which have been a subject for the present study were found to contain water from November to January. These being subject to constant biotic activity, were never found to support any higher kind of plants, not even such plants which would normally be free-floating or otherwise and tolerant to polluted waters. But, they often showed small or large flakes of matter originating from dead organic detritus mixed up with various Myxophyta, floating freely. The constant disturbance of water even perhaps seemed to retard the growth of non-flagellate planktonic life, since the water samples drawn during different times, usually at an interval of 15–20 days, mostly represented Flagellate-flora and fauna and several other small animal organisms.

The samples regularly collected for over a period of year (July 1956 to November 1957) from these ponds, particularly in the form of slimy matter loosely lying on the surface of the soil and water, on cursory observations showed a few diatoms-at a time 4–5 species. But when more careful examination was undertaken, some more forms were recorded. Curiously enough, all of the 5 ponds revealed a similar kind of flora both in quality and quantity. Moreover, many of the forms were constant but *Gomphonema parvulum* Kütz., *G.*-v. *micropus* (Kütz.) Cl., *G. montanum* Schum. v. *acuminatum* Mayer, *Nitzschia amphibia* Grun., *N.*-v. *acutiuscula* Grun. and *N. palea* (Kütz.) W. Sm., predominated the rest in one or the other pond. From further observations two striking facts were more or less apparent, *viz.*, (1) the diatom species

inhabiting the slimy matter were abundant in samples collected from borders of these wet situations (from soil surface), possibly representing the benthos of the loose soil, in contrast to isolated forms occurring in free-floating flakes of dead organic matter, and (2) the floristic makeup was of such individuals which scarcely exceeded 50 μ length, leaving a few habitually larger species of which only three were recorded. These latter forms then were invariably found to be isolated or rather stray. Even other diatoms which elsewhere are known to be of larger size were here either quite small or represented their smaller limits corresponding to the typical biotic pattern.

Of the diatoms studied, *Eunotia tschirchiana* O. Müll., was found to be interesting. The species observed here for a continuous period of 16-months tended to point out that besides some form-change, with diminution of the length its breadth hardly suffered any significant reduction. Thus, this diatom seemed to reveal its life-history feature to a certain extent.

Again, in a larger pond besides free-floating flakes of dead organic matter with Myxophyta, were found some isolated masses of *Pithophora*, *Sphaeroplea* and stray filaments of some *Oedogonium*. On these larger algæ, *Eunotia tschirchiana*, *Gomphonema parvulum*, *G.*-v. *micropus* and *G. lanceolatum* v. *affine* (Kütz.) Cl., were found to be also epiphytic particularly on the spots where encrustations of Calcium salts were present. The diatoms occurring thus, may be referred to Calciphilous group due to their this peculiar habit.

In this paper an illustrated taxonomical account is given particularly of the new taxa and new records for India and other illustrations are given to suggest any deviation occurring in the forms previously recorded. Further, under the individual diatoms besides their dimensions, field notes are also given and their distribution is suggested in the region of Ahmedabad leaving out the cases which have been dealt with elsewhere.

1. *Cyclotella meneghiniana* Kütz.

Diameter 11·5–14 μ, and striae 10 in 10 μ.

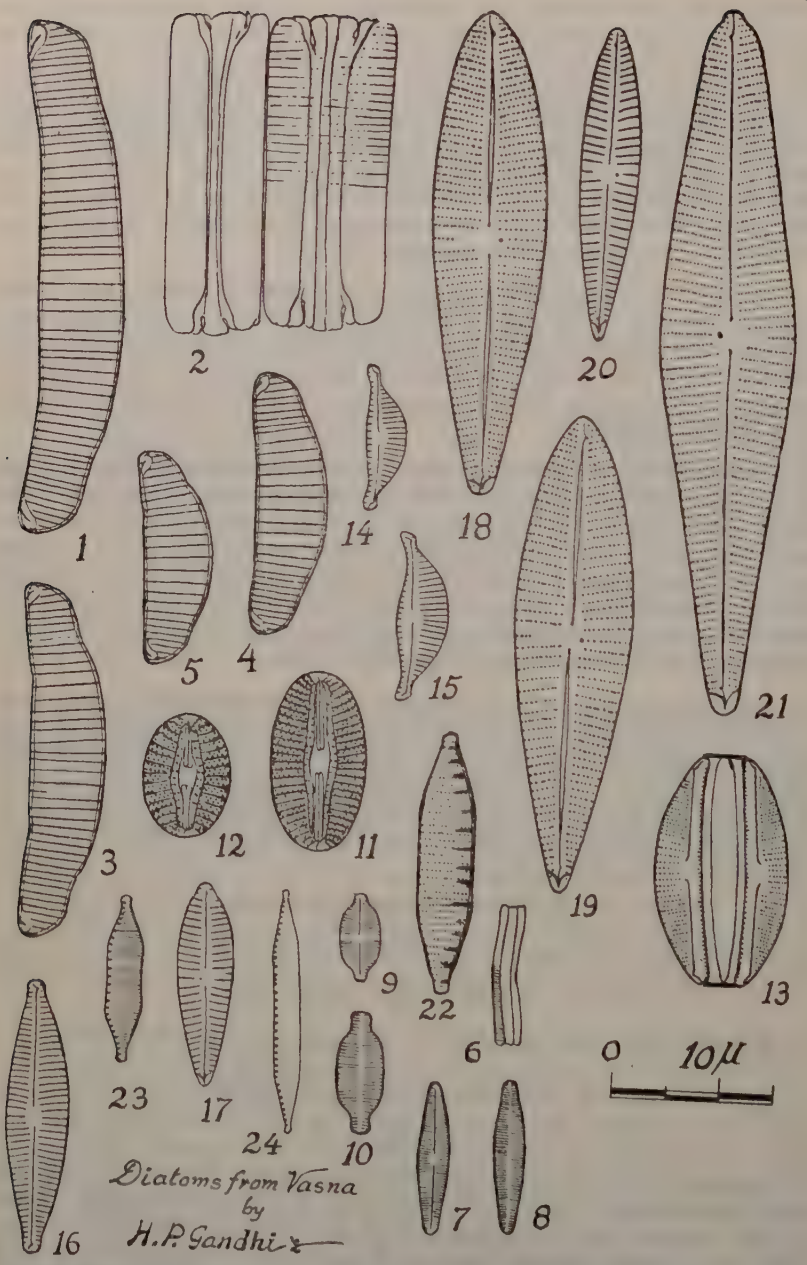
2. *Synedra ulna* (Kütz.) Ehr.

Length 110–165 μ, breadth 5–6·8 μ and striae 9–10 in 10 μ.

3. *Eunotia tschirchiana* O. Müll.

(Text-Figs. 1–5)

Schmidt, A., *Atlas Diat.*, 1874–1944, t. 382, f. 98–100; Hustedt, *Diat. Sunda-Exped.*, 1938, p. 173–74, t. 12, f. 23–29.—Frustules formed in short chains and rectangular in girdle view. Valves 20–50 μ long and 6–7·5 μ broad, slightly arcuate, dorsal side convex to strongly convex and ventral side more or less straight in the middle part with distinct bends towards the ends; ends gracefully constricted on the dorsal side with more or less produced obliquely truncate or subtruncate apices,



TEXT-FIGS. 1–24. Figs. 1–5. *Eunotia tschirchiana* O. Müll. Figs. 6–8. *Achnanthes minutissima* Kütz. Figs. 9–10. *Achnanthes exigua* Grun. Fig. 11. *Diploneis pseudovalis* Hust. Fig. 12. *Diploneis subovalis* Cl. v. *perminuta* (A. C).

Fig. 13. *Amphora veneta* Kütz. Figs. 14–15. *Cymbella thumensis* (A. Mayer. Hust. Fig. 16. *Cymbella fonticola* Hust. Fig. 17. *Gomphonema parvulum* v. *micropus* (Kütz.) Cl. Figs. 18–19. *Gomphonema montanum* Schum. v. *acuminatum* Mayer. Fig. 20. *Gomphonema lanceolatum* v. *affine* (Kütz.) A. Cl. Fig. 21. *Gomphonema subapicatum* Frit. & Rich. Fig. 22. *Nitzschia denticula* Grun. v. *rostrata* v. nov. Fig. 23. *Nitzschia microcephala* Grun. v. *elegantula* Grun. Fig. 24. *Nitzschia vasnaii* sp. nov.

Polar nodules small but distinct. Striae 6–9 in the middle and 9–14 in 10 μ at the ends, coarse, striae in the middle part distantly and irregularly formed and towards the ends gradually closely set and apparently regularly arranged.

TABLE I

Showing the typical dimensions as recorded

Length in μ	Breadth in μ	No. of middle striae in 10 μ	No. of end striae in 10 μ
20	7	7–8	9–13
25	7	8–9	9–14
28·5	6·6	7–9	9–12
28·0	6·6	7–9	9–14
34	7	7–8	9–13
50	7·5	6–8	10–14

This diatom closely agrees with the type as illustrated by Hustedt in the works cited hitherto, in the outline, somewhat in apices and the arrangement of striæ. However, the local specimens differ from the same in having more clearly constricted and produced ends and also in having denser striae both in the middle and terminal zones. Looking at the species capable of showing high degree of structural variations as being suggested by Hustedt in the said references—it is felt here that presently recorded deviations could be admitted within the type, they being local and of small order.

This diatom was commonly collected from marginal scum of the ponds but it also occurred as epiphytic on *Pithophora* and other large algae. These frustules formed short ribbons in which no two specimens were found of equal width. This species was also collected from other parts of Ahmedabad but more commonly from Chandola and Kankaria lakes and fountain reservoirs of Seth Sarabhai's garden. Fairly distributed in the locality.

4. *Achnanthes minutissima* Kütz.

(Text-Figs. 6–8)

Length 13·3–15 μ, breadth 2·3–2·5 μ and striae about 35 in 10 μ.

5. *Achnanthes exigua* Grun.

(Text-Figs. 9–10)

Length 9–12 μ, breadth 4·2–4·6 μ and striae 28–30 in 10 μ.

6. *Diploneis pseudovalis* Hust.

(Text-Fig. 11)

Length 13–17 μ, breadth 8·6–10 μ, costae 12–14 in 10 μ alternating with two rows of fine punctae or alveoli.

This species was seen in good numbers in marginal slime of the ponds as tiny beautiful forms. It was frequently seen in several other pools and ditches on the roadside outside the city proper. However, in all 20% of collections from Ahmedabad were found to contain it.

7. *Diploneis subovalis* Cl. v. *perminuta* A. Cl.

(Text-Fig. 12)

Cleve-Euler, A., *Diat. Schwed. Finn.*—III, 1953, p. 83, f. 654 A.—Valves 12·5–16 μ long and 7·5–9 μ broad, broadly oval or deceptively rounded. Raphe between the ribs, ribs slightly widened at the central nodule. Furrows broadly lanceolate, somewhat more dilated in the middle. Costae 12–13 in 10 μ, strongly conspicuous, radial at the ends, alternating with two rows of clearly discernible punctae.

This diatom also occurred with the above-named species but was marked out by its possession of broad furrows and strongly marked costae. It was found in good number both in the marginal slime and tangles of *Pithophora* or some Myxophyta. From other parts of Ahmedabad, it was collected from pools, garden reservoirs and constantly wet situations under the garden taps. Fairly well distributed in the region.

8. *Amphora veneta* Kütz.

(Text-Fig. 13)

Length 12–22 μ, breadth in girdle view 8–13 μ, striae in the middle 17–19 but towards the ends about 26 in 10 μ.

9. *Cymbella thumensis* (A. Mayer) Hustedt

(Text-Figs. 14–15)

Hustedt, *Diat. Balkan-Halbinsel*, 1945, p. 938, t. 42, f. 60–62; *Diat. norddeut. Seen*, 1950, p. 347, t. 37, f. 6–7.—Valves 12·5–15·2 μ long

and 3·8–4·7 μ broad, asymmetrical, semi-lanceolate, dorsal side strongly convex, ventral side more or less inflated in the middle, ends strongly constricted, capitate rounded or slightly obliquely rounded and somewhat ventrally bent. Raphe thin and straight or feebly curved and close to the ventral side; central pores dorsally bent and terminal fissures ventrally directed. Axial area very narrow; central area small and somewhat ventrally expanded. Striae 17–19 in 10 μ, distinct, radial on the dorsal side, very small marginal on the ventral side also sometimes obscure.

This species was found to be deceptive in its appearance since at the first sight it resembled some species of *Amphora*. However, the characteristic girdle view of *Amphora* was not seen. It was found to be a tiny beautiful species occurring in the marginal slime only. It was quite frequent in the samples collected at different times. Elsewhere in Ahmedabad, it occurred generally in larger pools soon after the rains. Not widely distributed.

Cleve-Euler regards this species as *C. parvula* Krasske (Cleve-Euler, A., *Diat. Schwed. Finn.*—IV, 1955, p. 128, f. 1180 *a–d*).

10. *Cymbella fonticola* Hust.

(Text-Fig. 16)

Hustedt, *Diat. Sunda-Exped.*, 1938, p. 422, t. 24, f. 21–24.—Valves 20–26 μ long and 5–5·6 μ broad, asymmetrical, lanceolate with feebly constricted produced ends. Raphe thin and straight, close to the ventral side with central pores dorsally bent and terminal fissures ventrally directed. Axial area very narrow; central area small and not well defined. Striae 16–19 in 10 μ, radial, at the ends somewhat perpendicular to the middle line, indistinctly punctate.

This species was found in very good number associated with other diatoms in light brown slimy matter or in films formed on the wet soil. From other parts of Ahmedabad also it was well represented in various pools, ditches and roadside water courses during the rains. In Chandola and Kankaria lakes it was found associated with rotting masses of vegetable matter. A fairly common type in the area.

11. *Gomphonema parvulum* (Kütz.) Grun.

Length 14–22 μ, breadth 5·5–6·6 μ and striae 16–18 in 10 μ.

12. *Gomphonema parvulum* v. *micropus* (Kütz.) Cl.

(Text-Fig. 17)

Hustedt, *Bacil.*, 1930, p. 373, f. 713 *c*; Geitler, L., *Formwechsel Diat.*, 1932, p. 41–67, f. 11–28; Cleve-Euler, A., *Diat. Schwed. Finn.*—IV, 1955, p. 178, f. 1269 *h–j*.—Valves 14–20 μ long and 5·6–6 μ broad, clavate-lanceolate with feebly constricted produced apex. Raphe thin and straight. Axial area narrow; central area somewhat unilaterally

expanded with an isolated stigma on the opposite side. Striae 12–14 in 10 μ slightly radial.

This diatom was found to be quite frequent in collections made during different times, both as epiphytic on larger algae and in the marginal slime. It occurred often with the species exhibiting several aspects as recorded by Geitler. From other parts of Ahmedabad, it was fairly well represented particularly in Chandola and Kankaria lakes and some fountain reservoirs. A fairly well distributed form in the region.

13. *Gomphonema parvulum* v. *subelliptica* Cl.

Length 12–14 μ, breadth 4·5–5 μ and striae 14–15 in 10 μ.

14. *Gomphonema montanum* Schum. v. *acuminatum* Mayer

(Text-Figs. 18–19)

Length 42–48 μ, breadth 10·5–11·5 μ and striæ 10–13 in 10 μ. This diatom slightly differs from the previously recorded forms in having somewhat less acute to feebly cuneate apex.

This species was found to be abundant in ponds during the post-rainy period. It occurred both in free state as well as attached to filaments of *Pithophora*, *Sphaeroplea* and *Oedogonium*, particularly on spots having encrustations of Calcium salts. Elsewhere in Ahmedabad, it was found in both permanent and semi-permanent bodies of water. A common type in the region.

15. *Gomphonema lanceolatum* Ehr.

Length 27–39 μ, breadth 7–8 μ and striae 11–13 in 10 μ.

16. *Gomphonema lanceolatum* v. *affine* (Kütz.) A. Cl.

(Text-Fig. 20)

Length 30–40 μ, breadth 5·6–7 μ and striæ 9–13 in 10 μ. The present illustration corresponds to Cleve-Euler's (*op. cit.*) Fig. no. 1280 *k–m*.

This diatom usually occurred in rather small number in these ponds in association of other species. It was found to form dense clusters around the Calcium encrusted parts of *Pithophora* filaments. Elsewhere in Ahmedabad also not well represented since in all 8–10% of samples showed its presence.

17. *Gomphonema subapicatum* Fritsch and Rich.

(Text-Fig. 21)

Length 48–62 μ, breadth 10–12·5 μ and striae 9–11 in 10 μ.

This species was collected as a very stray form in all these ponds, it was usually associated with dead and rotting vegetable matter. In Kankaria and Chandola lakes it was found to be rather abundantly

growing as also in certain garden reservoirs where the water remained undisturbed for a long time. Fairly distributed in the area.

18. *Nitzschia denticula* Grun. v. *rostrata* v. nov.

(Text-Fig. 22)

Valvae 24·7–32 μ longae atque 5·6–6 μ latae, lineares, apicibus cuneatis et rostratis. Carina tenuis, valde ex-centro, carina punctis elongato, 5–6 in 10 μ. Striae 14–15 in 10 μ, distincte punctatae, punctis circiter 20 in 10 μ. Typus lectus a H. P. Gandhi ad Vasna die 1956–57, et positus in herbario proprio auctoris sub numero slide no. AHM.—26.

Valves 24·7–32 μ long and 5·6–6 μ broad, linear with cuneate rostrate ends. Keel narrow and strongly excentric with keel punctae elongated 5–6 in 10 μ. Striae 14–15 in 10 μ, distinctly punctate, punctae about 20 in 10 μ.

This species agrees well with *N. denticula* Grun. (Hustedt, *Bacil.*, 1930, p. 407, f. 780), in the outline, keel punctae and the punctate striae. However, it differs from the type in having clearly cuneate-rostrate ends and somewhat less number of striae per 10 μ. It is, therefore, considered to be a new variety.

This diatom was usually found in a small number in the marginal slime of various ponds. It was a conspicuous form due to its prominent keel punctae and clearly punctate striae. It is not much known from other parts of the city.

19. *Nitzschia microcephala* Grun. v. *elengantula* Grun.

(Text-Fig. 23)

Length 12–15·2 μ, breadth 3–3·3 μ, keel punctae 14–15 in 10 μ and striae about 28–30 in 10 μ, often very fine.

20. *Nitzschia amphibia* Grun.

Length 14–33·3 μ, breadth 4·2–4·7 μ, keel punctae 7–9 in 10 μ and striae 16–18 in 10 μ.

21. *Nitzschia amphibia* v. *acutiuscula* Grun.

Length 23–34 μ, breadth 4–5–4·7 μ, keel punctæ 8–10 in 10 μ and striae about 18 in 10 μ.

22. *Nitzschia palea* (Kütz.) W. Sm.

Length 20–30 μ, breadth 2·5–3·6 μ, keel punctae 12–13 in 10 μ and striae not clearly marked but probably more than 30 in 10 μ.

23. *Nitzschia vasnaii* sp. nov.

(Text-Fig. 24)

Valvae 20–24·3 μ longae atque 2–2·3 μ latæ, lineares, marginibus parallalis atque apicibus elongatis cuneatis et breviter capitatis. Carina angustissima, ex-centro, carina punctis 16–18 in 10 μ, minuta. Striae tenuissimæ ac indistincte, probabiliter 40–45 in 10 μ. Typus lectus a H. P. Gandhi ad Vasna die 1956–57, et positus in herbario proprio auctoris sub numero slide no. AHM.—28.

Valves 20–24·3 μ long and 2–2·3 μ broad, linear with parallel sides and long cuneate shortly capitate ends. Keel very narrow, excentric with keel punctae 16–18 in 10 μ, minute. Striae very fine and indistinct, probably 40–45 in 10 μ.

This diatom does not agree with any of the known similar looking types, hence it is considered to be a new species.

This species was found in small numbers usually mixed up with slimy matter of the ponds or in pale brownish matter deposited on dead partially submerged leaves. Elsewhere in Ahmedabad it was seen in similar localities as well as in marginal slime of Chandola and Kankaria lakes, but in a small number.

24. *Nitzschia obtusa* W. Sm. v. *scalpelliformis* Grun.

Length 105–142 μ, breadth 7·6–8·5 μ, keel punctae 7–8 in 10 μ and striae about 30 in 10 μ, sometimes more.

This species was sparingly represented in these ponds in the marginal slime formed by dead vegetable matter, etc. From other parts of Ahmedabad it was found in all wet situations but in brackish waters it was more abundant and occasionally gregarious. A common type in the region.

Summary

The paper deals with the Diatom-communities of some five temporary ponds around Vasna village near Ahmedabad. From the periodic collection and observation of the material for a period of nearly 16 months it was noted that all these ponds which happened to be subject of constant biotic activity represented similar kind of Diatom flora both in quality and quantity. The Diatom species occurring in them usually were of small size representing a typical biotic-group and also they seemed to form a benthic group of the loose soil.

From these ponds in all 24 diatoms were collected representing 9 genera. Of these, 4 are new records for India, one species and one variety is considered to be new to the Science.

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STUDIES IN INDIAN ANTHOCEROTACEAE

III. The Morphology of *Anthoceros erectus* Kash. and some other species*

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In the earlier two papers of this series the morphology of *A. crispulus* (Mont.) Douin (Bharadwaj, 1950) and *A.* cf. *gemmulosus* (Hattori) Pandé (Bharadwaj, 1958) has been described. The present paper deals with the comparative morphology of a few other Indian species such as *A. erectus* Kash., *A. gemmulosus* and *A.* cf. *subtilis* St., and also includes a detailed discussion on the morphology of all the species of the genus studied so far by me as well as others such as Campbell (1918) and Bartlett (1928)—*A. fusiformis* Aust., and Proskauer (1948)—*A. punctatus* L. and *A. husnoti* St. To make the discussion as comprehensive as possible, evidences on the relevant features also have been adduced from my study of the dry, herbarium material of *A.* cf. *erectus* Kash., *A. punctatus* L., *A. husnoti* St., and *A. stableri* St.

The main purpose in studying the morphology of so many species of *Anthoceros* (Mich.) L., has been to elucidate the nature as well as the range of variations existing within the genus so that such studies could lead to recognition of criteria, suitable for the taxonomic treatment of the genus. I find that even after the yellow-spored species have been separated out of the old *Anthoceros* L. complex as *Phaeoceros* Prosk., the species of the remaining taxon, *Anthoceros* (Mich.) L., exhibit a very wide range of variations.

Older systematists such as Gottsche (1858) had tried to systematise the genus *Anthoceros* Linn., mainly on the basis of variations in the characters of pseudoelaters and divided it into 3 sections (*see* Proskauer, 1951, p. 331), *viz.*, *A. Elateres fibra spirali depicti*, B. *Elateres articulis elongatis sine fibra spirali*, *C. Elateres cellulis justo paullo longioribus articulatim compositi.*

Campbell (1907) made use of the criteria of Gottsche's section A for the creation of *Megaceros* and Stephani (1915) that of section B for *Aspiromitus* leaving section C as *Anthoceros*. Recently Proskauer (1951) has virtually reduced pseudoelaters as morphological non-entities in sections B and C of Gottsche (*l.c.*) and regrouped these sections as

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* Part of the work done during 1951–52 at Botany Department, Lucknow University, Lucknow.

genera *Anthoceros* (Mich.) L. em. Prosk., and *Phaeoceros* Prosk., abolishing Stephani's genus *Aspiromitus*. From my study of *A.* cf. *gemmulosus* (Bharadwaj, 1958) a complete four-celled pseudoelater has been reinstated as a definite morphological unit equivalent to a spore tetrad. Regarding this I can further state, on the basis of my study of other species whose account has been published or is to be published in this series, that within the genera *Anthoceros* and *Phaeoceros*, a complete pseudoelater is always homologous to a spore tetrad. In view of this finding I have found it difficult to dispense with the features exhibited by pseudoelaters within these genera as summarily as has been done by Proskauer (1951). Whereas he (Proskauer, 1951, Pl. II, Figs. 28–33) presented the sequence of variations in pseudoelaters of *Anthoceros* (Mich.) L., as an integrated series, I find that among the Indian species selected by me for study, some species uniformly have wide- and smooth-lumened, thin-walled pseudoelaters but others have narrow and dented-lumened, thick-walled pseudoelaters. Although I do not attach any undue significance to this variation at the present stage of my investigations, all the same this appears convenient to divide the long series of investigations into two stages, *i.e.*, (1) regarding species having wide- and smooth-lumened, thin-walled pseudoelater and (2) regarding species having narrow- and dented-lumened, thick-walled pseudoelaters.

With the completion of my comparative investigations on *A. erectus*, *A. gemmulosus* and *A.* cf. *subtilis* the study of the first stage concludes and for this reason at the end of this paper I have taken up detailed morphological comparisons and discussion for this set of species.

Material and Methods

A. erectus was collected by Pandé and Bharadwaj from Mussoorie (Luck. Univ. Herb. No. 4980) in 1946. The specimens were fixed in 90% alcohol and preserved in the usual mixture of 70% alcohol and pure glycerine. In the same way as detailed elsewhere (Bharadwaj, 1958), microtome sections were cut 4–6 μ thick and stained variously.

A. gemmulosus was collected from near Manibhanjan in Sikkim Himalayas by Pandé in 1940 (Luck. Univ. Herb. Nos. 4832, 4833) fixed *in situ* and preserved and treated as above.

A. cf. *erectus* was collected by Pandé and Srivastava (1952) from Pachmarhi (Luck. Univ. Herb. Nos. 3961, 4241).

A. punctatus was collected by Nicholson from Sussex, England, in 1909 (Schiffner, Exsiccati. Serie. XXII, No. 1088).

A. stableri was collected by Stabler in 1881 and 1906 from Westmorland, England (Schiffner, Exsiccati. Serie. XXII, No. 1090).

A. husnoti was collected by Nicholson in 1911 from Cintra, Portugal (Schiffner, Exsiccati. Serie. XXII, No. 1905).

A. cf. *subtilis* St. was collected by Pandé and Srivastava from the type locality of *A. subtilis*, Mangalore, S. India, in 1950 (Luck. Univ. Herb. No. 5001), fixed *in situ* in fluid and preserved as detailed above.

Dry, herbarium specimens were studied after they had been soaked in boiling hot water, long enough to stretch fully. The spore morphology was studied from spores directly mounted in dilute glycerine gradually brought to ± pure concentration as well as from acetolyzed spores after the method of acetolysis described by Erdtman (1933).

Taxonomic Descriptions

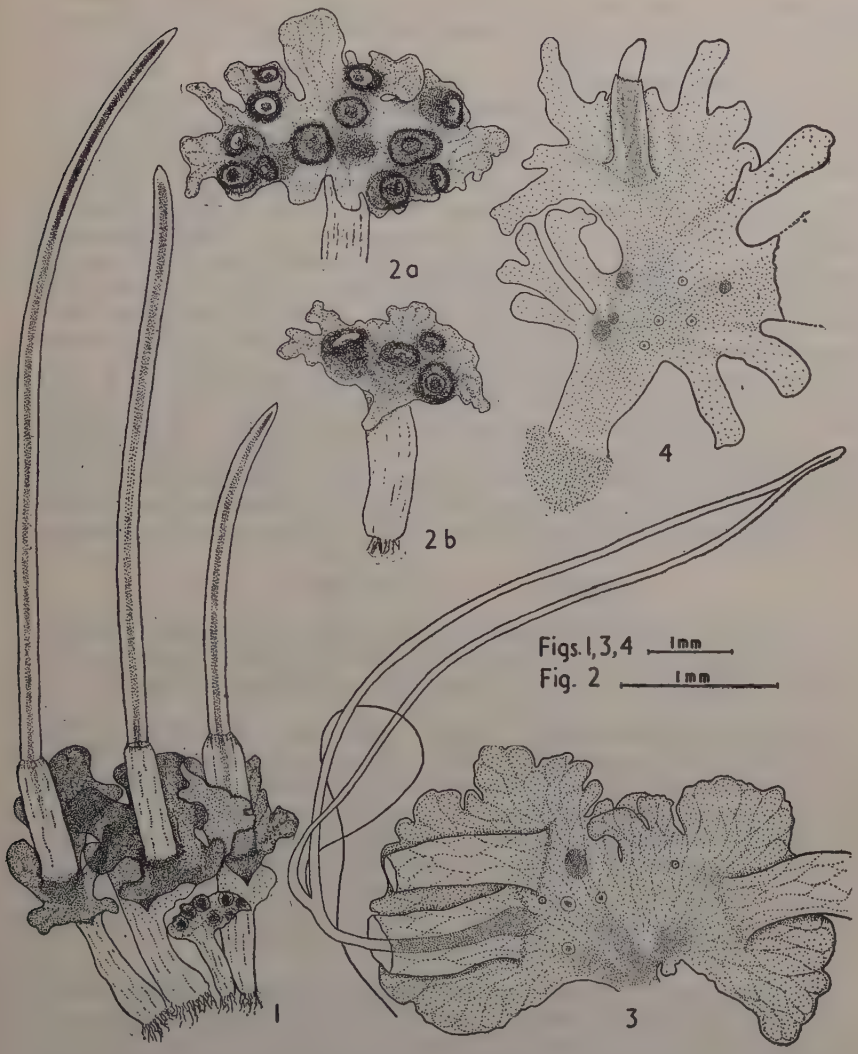
Anthoceros erectus Kash.

The plants forming the basis of the present study were collected from a single patch growing by the side of a drain near the main road in Landour, at Mussoorie.

Description.—Dioecious, erect, grouped into bunches just like flower bouquets; thallus thick, fleshy, spongy with a basal, thick, stalk-like structure expanding above into a lamina-like part (Text-Figs. 1, 2). Female thallus large, up to 10 mm. usually 5–7 mm. broad in the expanded part, stalk shorter (Text-Fig. 1) than in the case of male plants (Text-Fig. 2). Male plants usually smaller, 3–4 mm. high and 3–4 mm. broad at the top, growing mixed with the female plants. In female thalli the expanded lamina-like part is funnel-shaped or fan-like but it is mostly cup-like in male fronds. Margins in both the cases have deeply cleft segmentation of the lamina with longish lobes. The androecia are borne in and around the central region of the lamina.

In the androecia 12–30 antheridia have been counted in a chamber (Pl. XV, Fig. 4). Sometimes these are borne in two separate bunches in a single androecium. The stalk of a mature antheridium is 141 μ (mean of 18 counts) in length ranging from 103–166 μ. The body of mature antheridium is 145 μ (mean of 18 counts) ranging from 126–168 μ (Pl. XV, Fig. 5).

Sporophytes are borne towards the thicker central region of the expanded portion. The involucres may be sometimes partly gemminate, 2–4 mm. in length, cavernous and truncate. Capsules are 0·8 cm. to 3·5 cm. long, stomata numerous. Spores (Pl. XVI, Figs. 19–21) are spherical, black, reticulate with bold *muri* (ridges) which bear glossy, single *papillae* at irregular intervals, the ridges are broader on the distal face and narrower on the proximal face. The reticulum as well as the mesh-exine is covered with minute grana on both faces. The reticulum is uniformly developed on the distal as well as the proximal faces but for a narrow unsculptured stripe along the triradiate ridges which gives a distinctive appearance to the spore when viewed from proximal side appearing as if the triradiate ridges are white-bordered. The triradiate ridges do not extend up to the equator. The spore size (diameter based on mature spores inclusive of the *papillae* measured along one of the



TEXT-FIGS. 1–4. Fig. 1. *A. erectus*. A group of female and male fronds in natural position. Fig. 2. *A. erectus*. Two male fronds showing the receptacle-like expanded part borne on stalk with sunken androecia. Fig. 3. *A.* cf. *erectus*. Two fronds bearing capsules. Fig. 4. *A.* cf. *subtilis*. A frond exhibiting its longish nature and long lobes.

ridges) is 54 μ determined from 20 random counts, 10 each from 3 cm. and 0·8 cm. long capsules fixed in alcohol, range of size variation 49–59 μ. In glycerine preparations (5 years old specimens) the sport is 59 μ (average of 10 counts) ranging from 54–65 μ. Pseudoelaters are 4-celled, frequenttly 1 or 2-celled, branched; cells frequently humped,

thin-walled and collapsible on drying. The length of a complete pseudoelater is 143 μ (average of 18 random counts) ranging from 130–175 μ.

Prima facie, the diagnosis of *A. erectus* Kash. (Kashyap, 1915, p. 9), does not agree with the description of the specimen in question, in the size and sculpture of the spore. However, a critical appraisal of the description reveals that these differences are really non-existent and the two specimens are one and the same. In the original diagnosis (Kashyap *loc. cit.*) the spores size is given as 30–40 μ, but the figures include specimens, one of which is 52 μ and the other 46 μ, the latter apparently immature also. Likewise the sculpture interpreted and illustrated as granules (Kashyap, 1929, Pl. I, Fig. 3), leading to the term granulose (Kashyap, *loc. cit.*), represents really the mesh-work of a reticulum. It is apparently due to optical illusion, well known to palynologists when the ridges appear as channels and the meshes as granules. It is thus evident that my specimens are really *A. erectus* Kash. Kashyap's later (1932, p. 3) description of the spores of *A. erect*, based on specimens from Mussoorie, measuring 49–58 μ, minutely crenulate on the margin and with a fine reticulation on the convex side, is more accurate. The taxonomic description given here is more comprehensive and should serve to supplement the diagnosis given by Kashyap wherever necessary.

Anthoceros cf. *erectus* Kash.

In a preliminary survey of the Liverwort flora of Pachmarhi (Madhya Pradesh) Pandé and Srivastava (1952) reported some specimens as *A. erectus* Kash., which were subsequently examined by me.

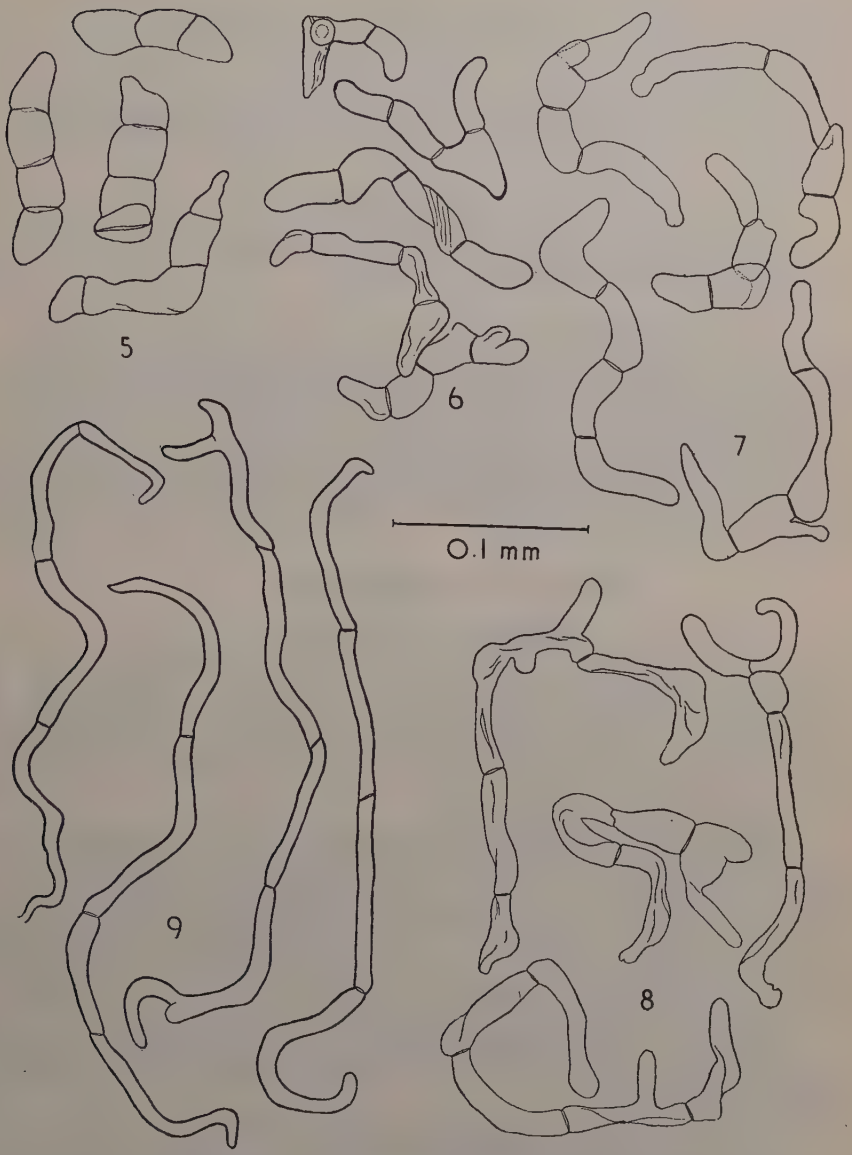
Description.—Monoecious, thallus prostrate, rosette-type with fronds 4–5 mm. long and as much or more broad posteriorly (Text-Fig. 3). Androecia small, having 12–22 antheridia, antheridial stalk two-tiered, antheridial body 84 μ long ranging from 76–90 μ. Capsules thin, 0·6–2·0 cm. long, involucre 1–2 mm. long. Spores irregularly reticuloid spinulate but for the narrow unsculptured stripes along the triradiate ridges (Text-Fig. 14), 46·5 μ (mean of 20 counts from water-soaked material) ranging from 43–51 μ. Pseudoelaters longish, measuring 220 μ (average of 18 counts), ranging from 184–260 μ.

As compared to *A. erectus*, the specimens from Pachmarhi show very marked differences in such respects as the disposition of the thallus, size of the antheridium, spore, pseudoelater, and mesh in spore sculpture, besides being monoecious. Thus it is not possible to consider them as *A. erectus* Kash., and I refer the Pachmarhi specimens as *A.* cf. *erectus* for the purposes of this and my other morphological papers.

Anthoceros stableri St.

Only male specimens were available.

Description.—Spongy, over 1 cm. long and with longish lobules similar to *A. husnoti* (Schiffner Exsiccati No. 1905). Size of antheridium in the Exsiccati specimens of *A. stableri* (Pl. XVI, Fig. 7) 94 μ (mean of



TEXT-FIGS. 5–9. Elaters. Fig. 5. *A.* cf. *subtilis.* Fig. 6. *A. erectus.* Fig. 7. *A. crispulus.* Fig. 8. *A. husnoti.* Fig. 9. *A. gemmulosus.*

18 counts) ranging from 69–100 μ. Proskauer (1948) has merged *A. stableri* with *A. husnoti* although according to him the size of the antheridial body in *A. husnoti* is 110–150 μ (mean 120 μ). As the material of *A. stableri* examined by me happens to be authentic,

collected by G. Stabler from Westmorland and probably the same as studied by Proskauer, in view of the differences in antheridial size I doubt very much if *A. stableri* and *A. husnoti* can be synonyms. I have preferred to maintain this an open question till something conclusive can be said on the basis of additional evidence regarding pseudoelaters and spores in *A. stableri* of Westmorland and the study of more material from this locality.

Anthoceros cf. *subtilis* St.

This material was collected by Dr. S. K. Pandé and Mr. K. P. Srivastava from Mangalore, S. India, in 1950.

Description.—Monoecious, thallus prostrate, rosette-type spongy, fronds 4–5 mm. long and as much as 3 mm. broad acquiring a longish look, margin deeply cleft giving rise to longish lobes (Text-Fig. 4), surface cells having one chloroplast each with a central group of pyrenoid bodies. Involucre 2–4 mm. long, cavernous, capsule 1·2–2 cm. long, pseudoelaters 105 μ long $\times$ 20 μ broad, spores 33–36 μ, black, reticuloid spinulate. Androecia small, sparse, raised as a mound, opening narrow, containing 4–8 antheridia, body wall 4-tiered. Antheridial body 102 μ (mean) ranging from 78–110 μ.

Morphological Descriptions

Anthoceros erectus Kash.

Gametophyte.—Dioecious (Text-Figs. 1, 2) erect, usually ♂ and ♀ growing intermixed. Rosettes incomplete. Male thalli expanded into a lamina-like part raised on robust stalks (Text-Figs. 2 *a*, *b*), rhizoids usually confined only at the basal end of the stalk-like part. Stalk cells elongated, thin-walled (Pl. XV, Fig. 5). Expanded portion deeply lobed, lobes with rounded outer margins.

Female thallus having a basal cylindrical stalk (not so prominent as in the male) expanding above into a lobed, lamina-like part (Text-Fig. 1), several cells thick towards centre gradually becoming thinner towards the margin. Mucilage chambers present only in the expanded part, *Nostoc* colonies present both in the stalk as well as the expanded part. Chloroplasts as in *A.* cf. *gemmulosus* (Bharadwaj, 1958) both in structure and organisation.

The thallus has several growing points, each with an apical cell. The segmentation of the apical cell follows the usual pattern as in other species of *Anthoceros*.

Sex Organs

Androecium.—Androecia scattered all over the expanded part of the male thallus, usually arranged in rows, the older being towards the centre and younger nearer the periphery. Evidently these develop in acropetal succession as usual in the genus. When mature they form deep cavities, mostly included in the thallus with their roof only slightly raised above the surface of the thallus. Superficially, the undehisced

androecia appear as prominent conical pustules. Dehiscence by a well-defined opening through which a tuft of antheridia seen arising from the centre of the cavity (Text-Figs. 2 *a*, *b*; Pl. XV, Fig. 4). Number of antheridia large, as many as 30 counted.

As is apparent from Plate XV, Fig. 4, the antheridia develop from a basal cushion (*b.c.*). The antheridia consist of a stalk and the body. The stalk is 4 cells thick but only two cells high (Pl. XV, Fig. 5). The cells of the upper tier in the stalk tend to swell out when mounted as usual in dilute glycerine (Pl. XV, Fig. 5). The antheridial wall is 4-tiered, the lower three consisting of elongated rectangular cells, while the topmost consists of broad-based triangular cells as in *A.* cf. *gemmulosus* (Bharadwaj, 1958).

Archegonium.—Archegonia develop, as usual, on the female thalli. The structure and development of the archegonium is of the usual type. The number of n.c. cells is up to 7. The ventral canal cell is practically the same size as the egg-cell. The canal cells have highly granular cytoplasm and are deeply chromatic. The neck is composed of 6 rows of cells capped by four large cover cells. Details of the archegonial development have already been given by Mehra and Handoo (1953) and my observations agree with theirs.

Sporophyte

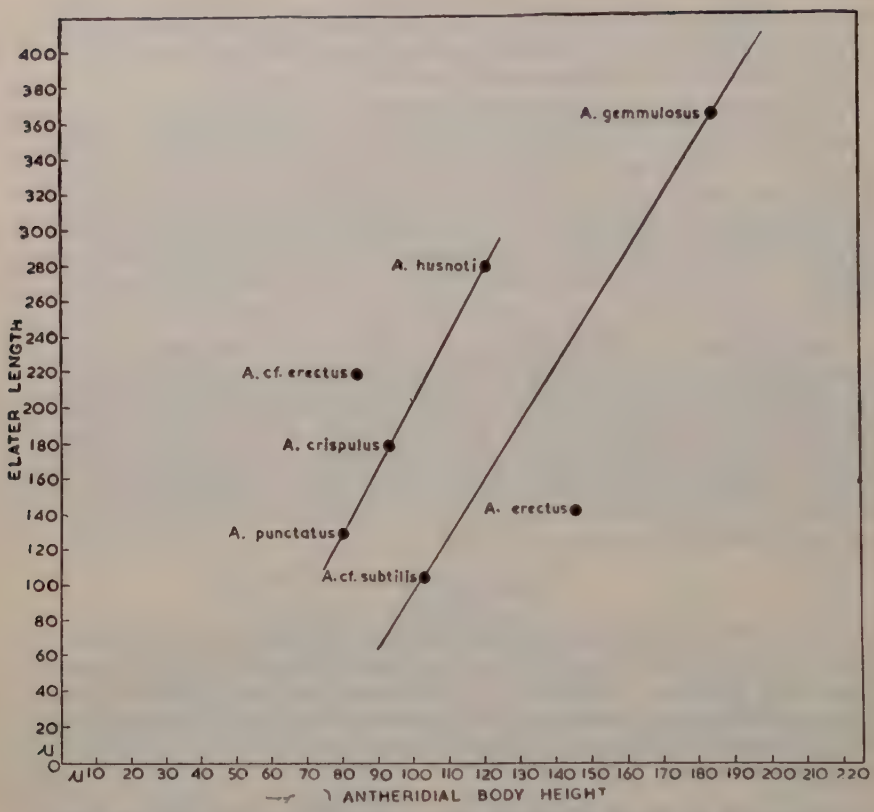
Foot.—The foot is comparatively smaller than that of *A.* cf. *gemmulosus*. It is well demarcated from the gametophytic tissue by a space filled with mucilage. Frequently a few small spherical cells may be included within the mucilage. The central cells of the foot are large and thin-walled while the peripheral ones are closely packed and elongated (Pl. XVI, Fig. 9).

Columella.—The endothecium is 4 cells thick and develops into the columella which as far as observed is only 4 cells thick in immature and small sporophytes (only two cells are seen in *l.s.*—Pl. XVI, Fig. 9). In the maturer regions of longer capsules, up to 16 cells thick columella has been seen. Mehra and Handoo (1953, Pl. II, Figs. 27, 28) have also figured a 16-celled columella.

Sporogenous tissue.—The sporogenous tissue, as far as observed, is always 1-cell thick. Mehra and Handoo (1953), however, observe that the sporogenous tissue of *A. erectus* frequently extends into the wall layers as a result of the transformation of some of the wall cells.

Normally the sporogenous layer alternately gives rise to spore- and elater-mother-cells as may be seen in archesporium a little above the foot. The spore-mother-cells are squarish or roundish while elater-mother-cells are rectangular and flattened. Both have great avidity for stains.

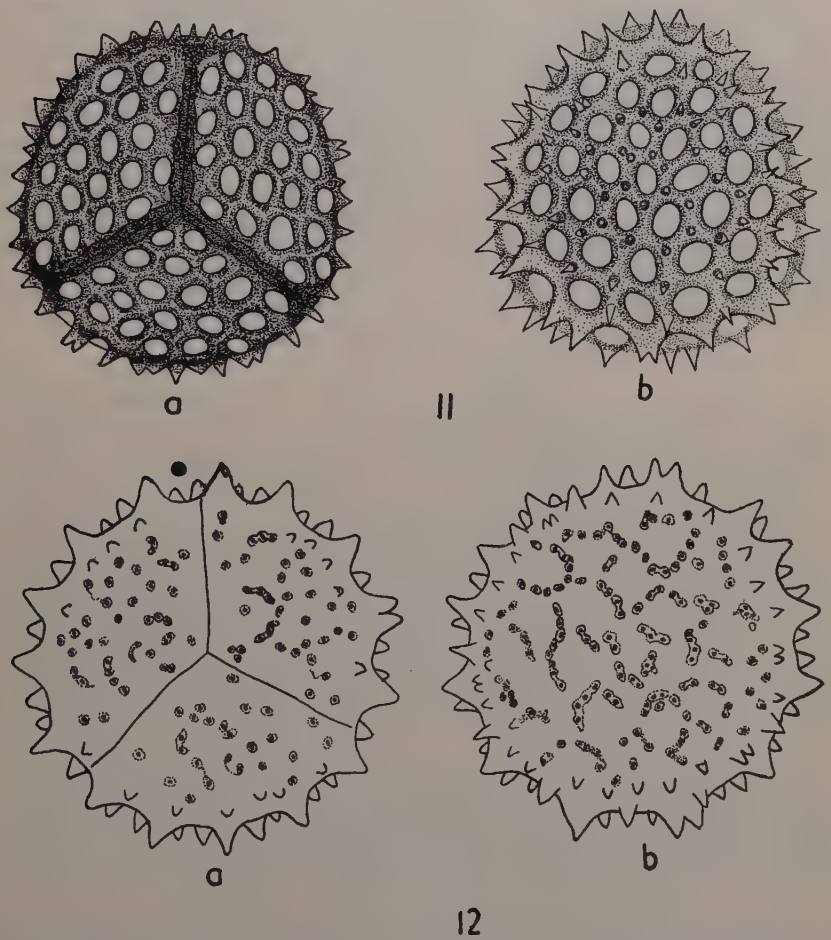
Spore.—The young spores occur in the form of tetrahedral spore-tetrads disuniting at maturity. In polar view, individual, mature spores are circular. The sporoderm consists of an outer, broad, sculptured



TEXT-FIG. 10. The comparative position of various species of *Anthoceros* on the combined basis of the antheridial body and elater sizes in Tables I and II.

layer (sexine) and an inner, very thin, non-sculptured layer (nexine). There does not appear to be any further differentiation within these two layers. In vertical section, the sexine has a wavy outer surface, the crests being of verrucose shape and disposition (Pl. XVI, Figs. 10, 11). It is also apparent that the sculpture is uniformly developed on all sides of the spore but for a narrow space along the two sides of the rays where the sexine is very thin (Pl. XVI, Figs. 10, 11). In mature spores this special feature results into the distinct demarcation of the rays from the rest of the sculpture (Pl. XV, Fig. 19) by a white stripe formed due to thinning of the exine in the region. In surface view the sculpture consists of a perfect reticulum with well-defined *muri* enclosing roundly squarish or polygonal meshes (Pl, XVI, Figs. 10, 21). The exine on proximal as well as distal faces is covered with minute *grana*. On both the faces the *muri* are peaked at intervals (Pl. XVI, Fig. 20). The peaks are more like *papillae*, small and transparent. The lips of the triletes rays are thick and raised. The rays terminate a little behind the equator of the spore (Text-Fig. 13; Pl. XVI, Figs. 19–21).

Pseudoelater.—Each pseudoelater is normally 4-celled, with the free end roundly tapering (Text-Fig. 6; Pl. XVI, Fig. 12). The development of pseudoelater from the pseudoelater-mother-cell was not closely followed but from the stages observed it is apparent that the details of development are fundamentally the same as in *A.* cf. *gemmulosus* (Bharadwaj, 1958). The wall of each pseudoelater-cell is normally unthickened and the lumen is wide, and smooth, containing granular cytoplasm. The pseudoelater cells are variously shaped. Mostly these are straight or slightly curved but not quite infrequently stickle-shaped or humped.



TEXT-FIGS. 11–12. Diagrammatic sketches of spores. Fig. 11. *A. punctatus* L. (*a*) proximal face, (*b*) distal face. Fig. 12. *A. gemmulosus* (Hattori) Pandé. (*a*) proximal face, (*b*) distal face.

Capsule wall.—The wall of the capsule is 4–5 cells thick including the outer, epidermal layer. On the epidermis, normal, functional stomata are richly and ± evenly distributed. The stomatal aperture is linear and is bounded by two guard cells. No abnormal or degenerated stoma were ever seen. The internal cells of the capsule wall are thin-walled and contain one or two chloroplasts each. The capsule dehisces into two valves which show marked twisting.

Anthoceros gemmulosus (Hattori) Schffn. and Pandé MS.

A detailed morphological investigation of *A. gemmulosus* was undertaken to elucidate any aspects in which this species might be morphologically different from *A.* cf. *gemmulosus.* It was found that in all features such as the shape and structure of the thallus, distribution, shape and organisation of the androecium, details of antheridial development including the budding of secondary antheridia; shape and organisation of the antheridium, details of archegonial development, development of embryo, foot, seta and capsule and structure of the capsule, *A. gemmulosus* agrees wholly with *A.* cf. *gemmulosus.* The only differences noted were the occurrence of solitary chloroplasts in thallus cells and longer pseudoelaters (Text-Fig. 9; Pl. XVI, Fig. 18) in *A. gemmulosus* about which reference has already been made earlier (Bharadwaj, 1958).

Anthoceros cf. *subtilis* St.

A comparative morphological investigation of *A.* cf. *subtilis* was undertaken regarding important features such as the structure of the thallus, distribution, shape and organisation of the androecium, shape and organisation of the antheridium and the structure of the capsule.

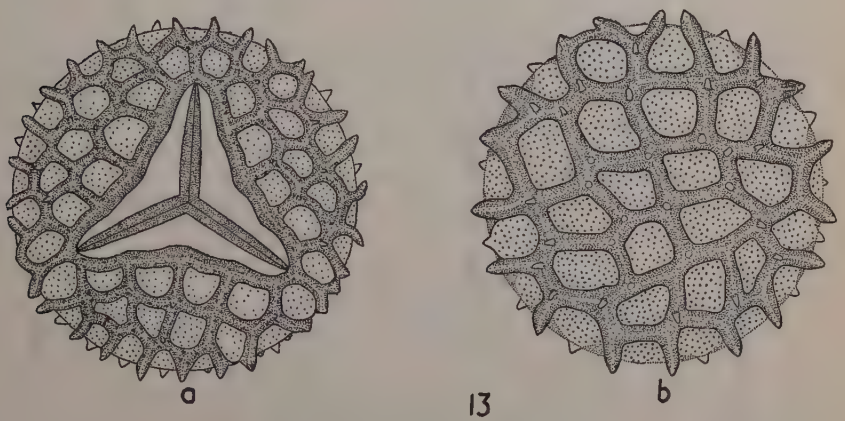
Thallus.—The species is monoecious and protandrous. The thalli (Text-Fig. 3; Pl. XV, Fig. 1) form prostrate rosettes. Fronds are anteriorly slender but posteriorly expanded. In the expanded part the striking aspect is the presence of long, marginal lobes (Text-Fig. 3). Each surface-cell contains one spongy chloroplast having a single group of pyrenoid bodies. In a *v.s.* the margin is rounded. Interior of the thallus is cavernous with apparently superimposed mucilage cavities. *Nostoc* colonies are present.

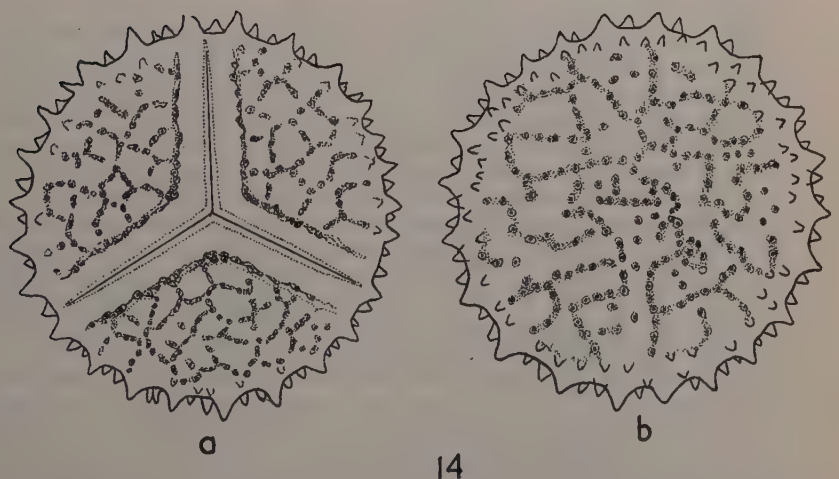
Androecium.—The androecia are small humps situated in the anterior part behind the capsule bases. Androecial opening is a well-defined pore (Pl. XV, Fig. 1). Antheridial bunch consists of young as well as mature, stalked antheridia of the usual type attached to a basal cushion as in other species. The basal cushion is small.

Capsule.—The foot is small and consists of a regular outer layer of palisade-like arranged cells as in other species. The columella is 16 cells thick and the capsule wall 4–5 cells thick. The cells of the capsular sheath have usually one chloroplast. The epidermis of the capsule is stomatiferous with functional stomata.

Spore.—The spores are circular in polar view, reticuloid spinulate, spinules sparser on proximal face (Text-Fig. 12), triradiate ridge not bordered, 33–36 μ in diameter.

Pseudoelaters.—Short but broad and extremely fragile pseudoelaters are characteristic of *A.* cf. *subtilis*. The free ends are broadly rounded. The pseudoelaters of *A.* cf. *subtilis* are the smallest among all the species considered here (Table II; Text-Fig. 5; Pl. XVI, Fig. 14).





TEXT-FIGS. 13–14. Diagrammatic sketches of spores. Fig. 13. *A. erectus* Kash. (*a*) proximal face, (*b*) distal face. Fig. 14. *A.* cf. *erectus*. (*a*) proximal face, (*b*) distal face.

TABLE I

Height of antheridial body

A. punctatus		*A.* cf. *erectus*		*A. crispulus*		*A.* cf. *subtilis*		*A. husnoti*		*A. erectus*		*A. gemmulosus*	
mean	range	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
80μ	50–90μ	84μ	68–90μ	92μ	72–105μ	102μ	78–110μ	120μ	110–150μ	145μ	126–168μ	184μ	150–210μ

TABLE II

Length of elater

A. cf. *subtilis*		*A. punctatus*		*A. erectus*		*A. crispulus*		*A.* cf. *erectus*		*A. husnoti*		*A. gemmulosus*	
mean	range	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
105μ	90–120μ	130μ	100–160μ	143μ	130–175μ	180μ	130–200μ	220μ	184–260μ	289μ	210–315μ	365μ	300–428μ

A. punctatus L.

The material available for study being dry, only the shape of the androecium and the androecial opening, and the spore morphology were studied to add to what has already been described by Proskauer (1948) and others.

Androecium.—Raised humps on the surface of the thallus (Pl. XV, Fig. 2) mark out the androecia. Opening well defined and bound by something that looks like a band or collar.

Spores.—In *A. punctatus*, *A. crispulus* and *A. husnoti* spores are similar in shape, sculpture and size. Roundly triangular or ± broadly oval with a well-defined triradiate tetrad mark having raised rays which extend almost to the equator (Text-Fig. 11 *a*; Pl. XVI, Fig. 28). Sculpture on the proximal face consisting of a perfect reticulum composed of low, undulating smooth *muri* which enclose subcircular lumina in the meshes. On the distal face *muri* of the perfect reticulum bearing prominent, single or bifid spines on their angular junctions (Text-Fig. 11 *b*; Pl. XVI, Fig. 29).

Pseudoelaters.—Four-celled with smooth, tapering ends (Pl. XVI, Figs. 15, 17). In *A. crispulus* as well, a re-examination in the light of these findings shows the complete pseudoelaters to be 4-celled and with smooth, tapering ends (Text-Fig. 7; Pl. XVI, Fig. 16).

Comparison of the Morphological Features in *Anthoceros* (Mich.) L.

The comparison of the morphological features in *Anthoceros* pertains mainly to the details studied in the three species, *viz.*, *A. crispulus*, *A.* cf. *gemmulosus* and *A. erectus*. For the corroboration of certain of my observations I have also drawn freely from the morphological details of *A. fusiformis* Aust., published by Campbell (1918), Bartlett (1928) and Proskauer (1951); *A. punctatus* and *A. husnoti* by Proskauer (1948, 1948 *a*); *A. erectus* by Mehra and Handoo (1953), and my own study of herbarium (fluid-fixed as well as dry) material of *A. gemmulosus*, *A.* cf. *erectus*, *A.* cf. *subtilis*, *A. punctatus*, *A. husnoti* and *A. stableri*. Comparisons have been restricted to only such characters where either corroboration of new findings has been needed or the characters show significant range of variation.

Thallus.—*A. crispulus*, as already described (Bharadwaj, 1950) is monoecious and so is *A.* cf. *subtilis*, *A.* cf. *erectus*, *A. punctatus* and *A. husnoti*. *A.* cf. *gemmulosus*, *A. gemmulosus* and *A. erectus* are dioecious.

The monoecious species are protandrous but the other species are strictly dioecious, the male and female fronds being more or less dimorphic. In not one single case out of numerous fronds of *A.* cf. *gemmulosus*, *A. gemmulosus* and *A. erectus*, examined by me, an androecium of any age, young or old, could be found on a frond bearing the sporophyte or *vice versa*. The androecia in these species are fairly

large and so there is hardly any chance of these escaping observations. As all the fronds of *A. gemmulosus* examined were united anteriorly in a rosette, a possibility of having missed the androecia from the female thalli due to decay of their older parts can also be excluded. Thus it can be stated that these species are strictly dioecious in nature. *A. punctatus*, *A. crispulus* and *A. husnoti* have a rosette forming prostrate thallus of which the fronds are posteriorly as broad as they are long and the margin is deeply notched. *A.* cf. *erectus* also develops similar, prostrate, rosette type of thallus but the margin is shallowly notched. *A.* cf. *subtilis*, *A.* cf. *gemmulosus* and *A. gemmulosus* also have prostrate rosette type of thallus but the fronds are longer than broad bearing elongated lobes along the margin. On the other hand *A. erectus* has erect fronds with a distinct stalk supporting a flattened lamina-like part breaking up into longish marginal lobes. The erect fronds in *A. erectus* are characteristically grouped into a sort of bouquet and thus are distinctive in organisation as compared to the prostrate, rosette forming thalli of other species. One might presume that these erect thalli arise as an ecological adaptation due to very moist habitat, particularly on the analogy of Proskauer's (1948) cultural experiments with *A. punctatus* and *A. husnoti* where under very moist conditions the fronds become exceedingly delicate and moist-etiolated, tending to become upright especially in the centre of the rosettes. But in *A. erectus* the erect thallus is no abnormality because the fronds are not etiolated, delicate or sterile as is usually found associated with the seemingly erect thalli in *A. punctatus* and *A. husnoti*. It seems that the erectness of the fronds in *A. erectus* is a feature of some consequence which does not seem to have arisen temporarily by the raising up of a prostrate frond.

In all the species the thalli are cavernous, *i.e.*, having schizogenous cavities filled with mucilage or in the older parts with air. But in *A. erectus*, the occurrence of basal stalk-like part which is compact as compared to the cavernous, posteriorly expanded part, is indicative that there can exist a manifestation of solidity in the fronds of some species of *Anthoceros* to a certain extent.

Androecium.—In all the species under consideration here, the androecium is a pouch-like chamber opening to the exterior by a well-formed pore, usually with a frill of tissue sometimes appearing as a collar round the pore (Pl. XV, Figs. 3, 4). The well-defined opening of the androecium was noted for the first time by Rink (1935) in *A. sampalocensis* but as it is evident now it occurs in all the species of *Anthoceros* studied so far. The frill round the opening is dropped out after some time.

The androecium is more or less superficial in *A. gemmulosus*, *A.* cf. *subtilis*, *A. punctatus* and *A. stableri* (Pl. XV, Figs. 1, 3, 6), *i.e.*, it appears as a conical (unopened) or rounded (opened) mound, most of which is raised above the general surface of the thallus. On the contrary in *A. erectus* the androecium is deeply (more than $\frac{1}{2}$) sunken in the thallus (Pl. XV, Fig. 5) with only a minor part appearing above the surface. Open androecia in *A. erectus* look like deeply scooped cavities or pouches.

Antheridium.—In all the species, the antheridium uniformly consists of a roundish oval or obovate body borne on a many cells high, 4 cells thick stalk. But in *A. erectus* the stalk is only two cells high.

The wall of the antheridial body is uniformly four-tiered in all the species considered by me which is in conformity with the findings of Proskauer (1948, 1951) for many other species of *Anthoceros*. To examine the tiered nature one should preferably examine dehisced antheridial bodies to avoid a confusion between the cell-walls in the body-wall and the lines dividing the segments of the antheridial contents. In all the species the cells of the apical tier cohere with each other in undehisced antheridia but are partially separated out during and after dehiscence and thus appear like the teeth of an operculum. In these cells of the apical tier, the median, incomplete vertical thickening in the outer wall of each cell seems to be mainly instrumental in curving the cells of the apical tier outwards when turged and thus opening the antheridia.

The origin of secondary antheridia in *A.* cf. *gemmulosus* and *A. crispulus* has already been described (Bharadwaj, 1958). In *A. erectus* and other species, the presence of a basal cushion subtending a number of young-to-old antheridia as in *A.* cf. *gemmulosus* suggests that the mode of origin of secondary antheridia in the former is very likely similar to that in the latter.

The size of the mature antheridial body (usually represented as the height only) varies within definable limits in a species. As it is difficult to ascertain whether an antheridium, before it has dehisced, is fully mature or not, I have always taken measurements of normal, dehisced antheridia. In Table I the size of antheridial body in different species are given. In Pl. XVI, Figs. 7, 8 the small antheridial body is that of *A. stableri* and the big one is of *A. gemmulosus*.

Embryogeny.—The position of the first septum in the oospore varies in different species. It is transverse in *A. crispulus* but vertical in *A. fusiformis*. According to Mehra and Handoo (1953) it is vertical in *A. erectus*. Nothing is known so far about the position of the first septum in *A.* cf. *gemmulosus*.

The development of a 4-tiered (16-celled) embryo, preceding the delimitation of the endothecium and the amphithecium, is known to exist in *A. crispulus* (Bharadwaj, 1950) and *A. fusiformis* (Bartlett, 1928, Fig. 3 A). In *A. erectus*, Mehra and Handoo (1953) have not observed anything like it and figure a two-tiered, 8-celled embryo (Text-Fig. 24) suggesting that the lower tier directly goes to form the foot and the upper tier the rest of the sporophyte without any further transverse divisions in them. Although such a condition is a deviation from the 4-tiered embryo observed in other species, it is certainly not improbable especially in view of the three-tiered embryo observed in *A.* cf. *gemmulosus* (Bharadwaj, 1958).

Foot.—In *A. crispulus* and *A. fusiformis* (Bartlett, 1928) the foot develops from the two, lower tiers comprising the hypobasal half in the 16-celled embryo. In *A. gemmulosus* and *A. erectus* the lone, hypobasal

tier forms the foot. The mature foot in all these species as well as *A. erectus* has a palisade-like outer layer of elongated cells surrounding the thin-walled inner cells.

Columella.—In all the three species investigated by me in detail as well as in *A. fusiformis* the endothecium wholly develops into the columella. In *A. erectus* the columella is only 4 cells thick in the younger regions but 16-celled in mature parts as far as observed. In *A. crispulus* it is uniformly 16 cells thick in young as well as in older parts and in *A.* cf. *gemmulosus* it is 16 cells thick in the younger parts but is 36–49 cells thick in the older, maturer regions of the capsule. In *A. fusiformis*, the columella is normally 16 cells thick but in older specimens it is more than 16 cells thick.

Sporogenous tissue.—The mature sporogenous tissue is uniformly one cell thick in *A. crispulus*, *A. erectus* and *A. fusiformis* (Campbell, 1928). According to Bartlett (1928) all the black-spored species of *Anthoceros* L., investigated by her, contain one cell thick sporogenous tissue, without any periclinal divisions occurring in the archesporial cells. But in *A.* cf. *gemmulosus* and *A. gemmulosus* usually one or two periclinal septa are developed in the archesporial cells, just above the setal region and the sporogenous tissue becomes two or three cells thick. In the apical region, above and around the columella-tip, the sporogenous tissue is many-layered in *A. gemmulosus* and *A.* cf. *gemmulosus*.

Spores.—In the species considered here the spores have either reticulate or reticuloid spinulate sculpture which is either in contact with or separated from the triradiate ridge by an unsculptured stripe on proximal face. In some species, *e.g.*, *A. punctatus*, *A. crispulus*, *A. husnoti* (Text-Fig. 11, Pl. XVI, Figs. 28, 29) the proximal faces have a smooth reticulum which is in contact with the rays of the triradiate mark but in *A. erectus* (Text-Fig. 13; Pl. XVI, Figs. 19–21) an unsculptured stripe of thin exine separates the reticulum from the rays on proximal face. In *A.* cf. *subtilis* (Text-Fig. 12; Pl. XVI, Figs. 26, 27) and *A. gemmulosus* the proximal faces lack the reticulum and are spinulate. In *A.* cf. *erectus* the reticuloid spinulate sculpture is separated from the triradiate ridge by an unsculptured stripe (Text-Fig. 14; Pl. XVI, Figs. 22, 23). Similar spores are also described by Proskauer (1954, 1958) for *A. fusiformis* Aust., *A. caucasicus* St. and *A. mandoni* St.

Pseudoelaters.—The origin and development of the pseudoelater in *A.* cf. *gemmulosus* has already been described in detail (Bharadwaj, 1958) concluding thereof that each pseudoelater is a compound, normally 4-celled structure, homologous to a spore-tetrad. In *A. erectus* and *A. crispulus*, from a number of relevant stages observed, it is evident that the pseudoelaters in these species as well, are 4-celled and thus homologous to spore-tetrads. The pseudoelaters in *A. punctatus* (Pl. XVI, Fig. 15), *A. crispulus* (Text-Fig. 7; Pl. XVI, Fig. 16), *A. husnoti* (Text-Fig. 8; Pl. XVI, Fig. 17), *A.* cf. *subtilis* (Text-Fig. 5; Pl. XVI, Fig. 14), *A.* cf. *erectus* (Pl. XVI, Fig. 13), *A. erectus* (Text-Fig. 6; Pl. XVI, Fig. 12) and *A. gemmulosus* (Text-Fig. 9; Pl. XVI, Fig. 18) are all normally four-celled as in *A.* cf. *gemmulosus*. Occasionally a pseudoelater may bear

an additional appendage (branch), normally as an aseptate extension of a pseudoelater-cell (Pl. XVI, Fig. 19).

In so far as I have studied the elaters in these species, *in situ* as well as in dispersed condition, there is no evidence of a *network* of sterile cell as has been supposed by Goebel and Suessenguth (1927) to occur in *A. punctatus*. As the complete, 4-celled, individual pseudoelaters, in all the species of *Anthoceros*, examined by me, have smooth, rounded, broad to tapering ends there is no evidence in these species for the existence of " a haphazardly constructed three dimensional girder system linked by secondary contacts " as interpreted for *Anthoceros* (and *Phaeoceros*) by Proskauer (1951, p. 337). The morphology of the pseudoelater (*i.e.*, an independent, complete individual developed from an archesporial cell, homologous to a spore-mother-cell—Bharadwaj, 1958) also does not support such a contention. In a carefully prepared, slide of mature spores and pseudoelaters one would seldom fail to see a number of complete pseudoelaters among many one-, two- or three-celled pieces of broken pseudoelaters. The breakage of pseudoelaters is a natural consequence of their compound nature.

In anacrogynous Jungermanniales the elater-mother-cell and the spore-mother-cell are homologous. In *Reboulia hemispherica* (Haupt, 1921, p. 449) as well as *Fossombronia cristula* (Haupt, 1920, p. 20) each elater- or spore-mother-cell develops into an elater or a spore tetrad respectively as in *Anthoceros*. But in *Marchantia* (Durand, 1908, p. 331) the elater-mother-cell is differentiated a few generations earlier than the spore-mother-cell so that an elater is homologous to a row of spore tetrads. Yet we call homologous structures in *Anacrogynae* and *Anthoceros* (? Anthocerotales) by different names, *i.e.*, ' elater ' and ' pseudoelater ' respectively or designate heterologous structures in *Anacrogynae* and *Marchantia* (? Marchantiales) as ' elaters '. This situation is rather anomalous.

It seems that the term pseudoelater in Anthocerotales came to be used for two reasons, firstly that here the identity of a complete elater, equivalent to a spore tetrad, was not established for a long time and secondly the occurrence of one- to many-celled pieces of sterile cells lent an inconsistency to their composition and thereby difficulty in their definition. Now, that a complete pseudoelater is morphologically defined being an unit equivalent to a spore tetrad, I prefer to term such an unit, henceforth, as an elater.

In some species of *Anthoceros* considered here, the breakage of the elaters is little, thus liberating many complete elaters as in *A. gemmulosus*, *A.* cf. *erectus* and *A. husnoti*, but in others, *e.g.*, *A.* cf. *subtilis*, *A. punctatus*, *A. crispulus* and *A. erectus* the elaters usually break up into 1–3-celled pieces which can be termed if must, as pseudoelaters or preferably as elater-cells, each cell individually equivalent to a spore.

There is no variation in the structure of the elaters among the species considered here (Text-Figs. 5–9). The lumen of the elater-cells is wide, smooth and the walls are thin and thus collapsible on drying.

The width of the elater-cells varies inversely as the length. Quantitatively the length of elater varies substantially between various species and because within a species the elater-length varies only within definable limits it appears to be a character of diagnostic value. In Table II the lengths of elaters in different species are given.

Discussion

There are a number of morphological features in the species of *Anthoceros* investigated by me, which are uniformly characteristic of all the species but in certain other features these species differ among themselves to a smaller or greater degree.

All the species considered here agree in the following features:—

They have cavernous thalli. Each thallus cell has one or more chloroplasts which have a central group of pyrenoid bodies. The androecia open by a well-defined pore. In the antheridial bunch, the primary antheridia are borne on primary basal-cells, one on each, the latter giving rise to secondary antheridia and expanding themselves into a marginally meristematic basal cushion. The antheridial body has a wall of four vertical tiers of cells with the cells of the apical tier functioning as in an operculum. The archesporium originates from the inner part of the amphithecium, foot from the lower half of the oospore and seta and capsule from the upper half. The foot is externally lined by a layer of palisade-like cells and thus is clearly delimited from the thallus tissue. The columella is smooth. Spores are tetrahedral, black, with their distal face reticulate with peaked *muri*. The elaters are 4 cells long, each elater-cell having wide and smooth lumen with its thin walls collapsing on drying.

The species of *Anthoceros* under consideration here also differ among themselves. The variations, detailed in the preceding pages can be categorised either as qualitative (morphological) or as quantitative variations. The qualitative variations are in the sculpture of the spore, in the disposition of the thallus and the shape of its fronds, the plane of the first division of the oospore and the number of tiers in the mature embryo preceding the delimitation of foot, endo- and amphithecium. The quantitative variations are in respect of the size of the thallus, number of antheridia in an androecium, number of tiers in the antheridial stalk, height of the antheridial body, length of capsules, size of foot, thickness (in number of cells) of the columella and the length of elaters.

Qualitative variations.—Among the qualitative variations the difference in sculpture on the proximal faces of spores appears to be most distinctive and easily observable. Not only that, as far as I have been able to determine, the various types of sculptural patterns met with are constant. It is for this reason that between *A. punctatus*, *A. crispulus* and *A. husnoti* or *A.* cf. *subtilis* and *A. gemmulosus* or *A. erectus* and *A.* cf. *erectus* in spite of marked differences in respect of some other characters the spore-sculptural type is constant.

On the basis of the sculptural variations on the proximal faces in spores, these species form four main groups.

(A) Spore with reticulum in contact with the triradiate mark, spore size 45–52 μ—*A. punctatus*, *A. husnoti*.

(B) Spore with spinules or proximal face, spore size 33–36 μ—*A.* cf. *gemmulosus*, *A. gemmulosus*, *A.* cf. *subtilis*.

(C) Spore with reticulum separated from the triradiate mark by an unsculptured stripe, spore size 49–59 μ—*A. erectus* Kash.

(D) Spore with reticuloid spinules separated from the triradiate mark by an unsculptured stripe, spore size 43–51 μ—*A.* cf. *erectus* Kash., *A. fusiformis*, *A. caucasicus*, *A. mandoni*.

The prostrate rosette type of thallus with fronds, as long as posteriorly broad, is the characteristic thallus of *A. punctatus*, *A. crispulus*, *A. husnoti* group. In *A.* cf. *subtilis*, *A.* cf. *gemmulosus* and *A. gemmulosus* group the thalli, though of prostrate rosette type, have fronds which have a marked tendency to be progressively linear in more elaborate species. In *A.* cf. *erectus* the thalli are prostrate rosette type with shallowly notched margin but in *A. erectus* the thalli are erect with longish lobed margin of the fronds. It is apparent that each of these variations though not strikingly different from the others are individualistic tendencies for each of the spore-group species.

The plane of the first division of the oospore in *Anthoceros* spp. seems to be a qualitative distinction of definable significance. We already know that in *A. crispulus* the first septum is transverse and in *A. erectus* as well as *A. fusiformis* it is vertical. This difference, in view of the now known qualitative distinction between the spore types of the three species, is of importance. Likwise the occurrence of 4-tiered mature embryo in *A. crispulus*, 3-tiered mature embryo in *A.* cf. *gemmulosus*, 2-tiered mature embryo in *A. erectus* and 4-tiered mature embryo in *A. fusiformis* (Bartlett, 1928) go hand in hand with the differences in the sculpture of the spore in these species.

From what has been discussed above it is evident that differences in the spore sculpture are associated with comparable qualitative differences in several other morphological features. Thus in respect of qualitative variations (primarily the spore sculpture) these species can be grouped into the following way:—

I. A. punctatus group.—Spores reticulate, perfect reticulum in contact with triradiate ridges, spore size 45–52 μ. Thallus prostrate rosette type with fronds posteriorly broad and having lobed margin, androecium superficial, first division of the oospore transverse, mature embryo 16-celled (incl. *A. punctatus*, *A. crispulus*, *A. husnoti* and *A. neesii* Prosk.

II. A. gemmulosus group.—Spores reticuloid spinulate on distal face only and spinulate on the proximal face, spore size 33–36 μ. Thallus prostrate rosette type with fronds narrow and marginal lobes linear,

androecium superfecial, first division of the oospore -?, mature embryo 12-celled (incl. *A.* cf. *subtilis*, *A.* cf. *gemmulosus* and *A. gemmulosus*).

III. A. erectus group.—Spores perfectly reticulate reticulum papillate on distal as well as proximal sides but separated from the triradiate ridges by narrow unsculptured stripes along both sides of the rays, spore size 49–59 μ. Thallus erect, fronds with longish lobes, androecium sunken, first division of the oospore vertical, mature embryo 8-celled (incl. *A. erectus*).

IV. A. cf. *erectus group.*—Spores reticuloid spinulate on distal as well as on proximal side, separated from the triradiate ridges by a narrow unsculptured stripe along both sides of the rays, spore size 43–51 μ, thallus prostrate rosette type with posteriorly broad fronds, first division of oospore vertical and mature embryo 16-celled (incl. *A.* cf. *erectus*, *A. fusiformis*, *A. caucasicus*, *A. mandoni*).

Quantitative variations.—Among the quantitative variations all are not of equal significance. Thus the size of the thallus, the number of antheridia in an androecium, or the length of the capsule appear to be of less consequence because the range of variation within these is apparently undefinable (compare Campbell, 1924 on *A. fusiformis* and Proskauer, 1948, on *A. punctatus* and *A. husnoti*). These characters appear to be easily affected by changes in ecological factors and/or the length of growing period and are apparently plastic.

At the same time there are some variations such as the occurrence of two cells or many cells high antheridial stalk or one- to many-layered archesporium in various species which may not have come up as a consequence of ecological variations. But these characters being not easily observable are of little practical utility for general taxonomic study. However, there are also some characters, in which quantitative variations are definable and which have so far not been shown to be easily affected by external factors of growth and nutrition and yet are easily observable such as the size of the mature antheridial body, usually represented by the height (Table I) and the length of complete elaters (Table II). It is apparent from the tables that the averages of these variations, in spite of wide limits, usually do not merge between species that have similar spore type, *e.g.*, between *A. punctatus*, *A. crispulus* and *A. husnoti*.

In Tables I and II the variations are tabulated in an ascending series, *i.e.*, from smallest to the biggest. In each of these tables it appears as if all the species considered here form a normal integrated series of elaboration. But if the two tables are compared it becomes evident that the order of the species in both the tables is not the same meaning thereby that in regard to the combined elaboration of these two variables, these species of *Anthoceros* do not form an integrated series. To enable a fuller appreciation of this differential behaviour, the readings for each species in Tables I and II have been plotted on a graph (Text-Fig. 10) where one can see that these species do not tend to fall into one line, straight or even a uniform curve, but are scattered all over

indicating thereby that all these species do not represent an integrated series. The only straight lines possible are those obtained by joining the points representing the group of species having similar spore sculpture. Thus *A. punctatus*, *A. crispulus* and *A. husnoti* fall in a straight line on which no other species falls. Likewise the points of *A.* cf. *subtilis* and *A. gemmulosus* when joined together form yet another line. As *A. erectus* and *A.* cf. *erectus* are the lone representatives of their groups no line can be drawn yet their position being away from the lines of *A. punctatus* and *A. gemmulosus* groups as well as from each other clearly suggests their distinctive nature as compared to others. To me these lines signify evolutionary sequences in which the species of *Anthoceros* are tending to evolve or have evolved. In a way Text-Fig. 10 confirms that the grouping of the species of *Anthoceros* on the basis of variation in spore sculpture is natural. It is apparent that the evolution of species in *Anthoceros* has been progressing independently in a number of groups so that even if all the species appear superficially to be more or less alike these do not have so much in common.

Conclusion

From the detailed investigation of a large number of species of *Anthoceros* possessing thin-walled, wide- and smooth-lumened elaters it is evident that these species are not units of one uniform evolutionary series but instead they can be grouped together separately into four main groups according to the type of their spore sculpture. The fact that the spore is a morphologically conservative organ and so the variation in its sculptural patterns are of phyletic value, is substantiated by other morphological (qualitative) variations associated with the spore characters. The quadripartite grouping of these species of *Anthoceros* is further confirmed by the comparative differential behaviour in the elaboration or otherwise of the size of the antheridial body and the length of elaters in these species.

Summary

1. Taxonomic descriptions of *A. erectus*, Kash., *A.* cf. *erectus*, *A. stableri* St. and *A.* cf. *subtilis* St. have been included. In the taxonomic details of *A. erectus* Kash. the diagnosis given by Kashyap has been elaborated. Important taxonomic details of a specimen listed as *A. erectus* from Pachmarhi (Pandé and Srivastava, 1952), and referred here as *A.* cf. *erectus* have been given showing thereby that the latter differs from *A. erectus* markedly. Some details given about *A. stableri* indicate that it may not be a synonym of *A. husnoti*. Taxonomic details of *A.* cf. *subtilis* have been given.

2. Morphology of *A. erectus* is described.

3. Corroborative morphological facts from a study of *A. gemmulosus*, *A.* cf. *subtilis* and *A. punctatus* have also been described.

4. The morphological details as revealed from the detailed study of *A. crispulus* (Bharadwaj, 1950), *A.* cf. *gemmulosus* (Bhardwaj, 1958),

A. erectus and the comparative study of *A. gemmulosus*, *A.* cf. *subtilis* and *A. punctatus* by me as well as those from the publications of other authors on *A. fusiformis*, *A. punctatus* and *A. husnoti* have been compared.

5. The following has been discussed and concluded:—

(*a*) All the species considered here agree in respect of a number of qualitative morphological characters.

(*b*) It has also been discovered that among these species qualitative as well as quantitative variations exist. Qualitative variations occur in the shape and disposition of the thallus, the plane of the first wall in oospore division, the occurrence of 8–12- or 16-celled embryo preceding the delimitation of endo- and amphithecium, and in the spore sculpture. On the basis of these variations, chiefly the last one, the species are grouped as follows:—

I. *A. punctatus* group (incl. *A. crispulus*, *A. husnoti*, *A. neesii*).

II. *A. gemmulosus* group (incl. *A.* cf. *subtilis*).

III. *A. erectus* group.

IV. *A.* cf. *erectus* group (incl. *A. fusiformis*, *A. caucasicus*, *A. mandoni*).

Among the many quantitatively varying characters the height of the antheridial body and the length of an elater have been considered as most important for taxonomic purposes. The comparative manifestation of these two variables in different species segregates them into a number of distinct evolutionary sequences corresponding to the spore sculptural groups given above.

It has been concluded that the spore sculpture is of paramount importance for the segregation of taxonomic group in this section of the genus, and that the differences in the spore sculpture are also equally manifest by variations in the other morphological characters within *Anthoceros* spp., containing thin-walled, wide- and smooth-lumened elaters.

Acknowledgement

I am thankful to Prof. S. K. Pandé for the material, and critical suggestions during the preparation of this paper. I am also obliged to Prof. S. N. Das Gupta, the then Head of the Botany Department, Lucknow University, for research facilities and encouragement.

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EXPLANATION OF PLATES XV and XVI

PLATE XV

FIG. 1. *A.* cf. *subtilis.* Part of a frond showing longish lobules and a few bulging androecia (*a.*), ×6·2.

FIG. 2. *A. punctatus* (Schffn. Exsicc.). Part of a frond showing androecia (*a.*) and androecial aperture (*p.*), ×42·5.

FIG. 3. *A. stableri* (Schffn. Exsicc.). Part of a frond showing androecia (*a.*) and androecial aperture (*p.*), ×42·5.

FIG. 4. *A. erectus.* Vertical section through a male plant showing the erect, compact stalk and a sunken androecium with its narrow mouth. The antheridia are in large number and borne on a basal cushion (*b.c.*), ×100.

FIG. 5. *A. erectus.* A group of dehisced antheridia, ×175.

FIG. 6. *A gemmulosus.* Male plant, ×5·2.

PLATE XVI

FIG. 7. *A. stableri.* Dehisced antheridium, ×175.

FIG. 8. *A.* cf. *gemmulosus.* A dehisced antheridium, ×175.

FIG. 9. *A. erectus.* L.s. of sporophytes showing foot, columella and archesporium, ×115.

FIGS. 10–11. *A. erectus.* L.s. through maturing spore tetrads, ×500.

FIG. 12. *A. erectus.* A complete elater, ×175.

FIG. 13. *A.* cf. *erectus.* A complete elater, ×175.

FIG. 14. *A.* cf. *subtilis.* An incomplete (3-celled) elater, ×210.

FIG. 15. *A. punctatus.* A complete elater, ×210.

FIG. 16. *A. crispulus.* Two complete elaters, ×210.

FIG. 17. *A. husnoti.* A complete elater, ×210.

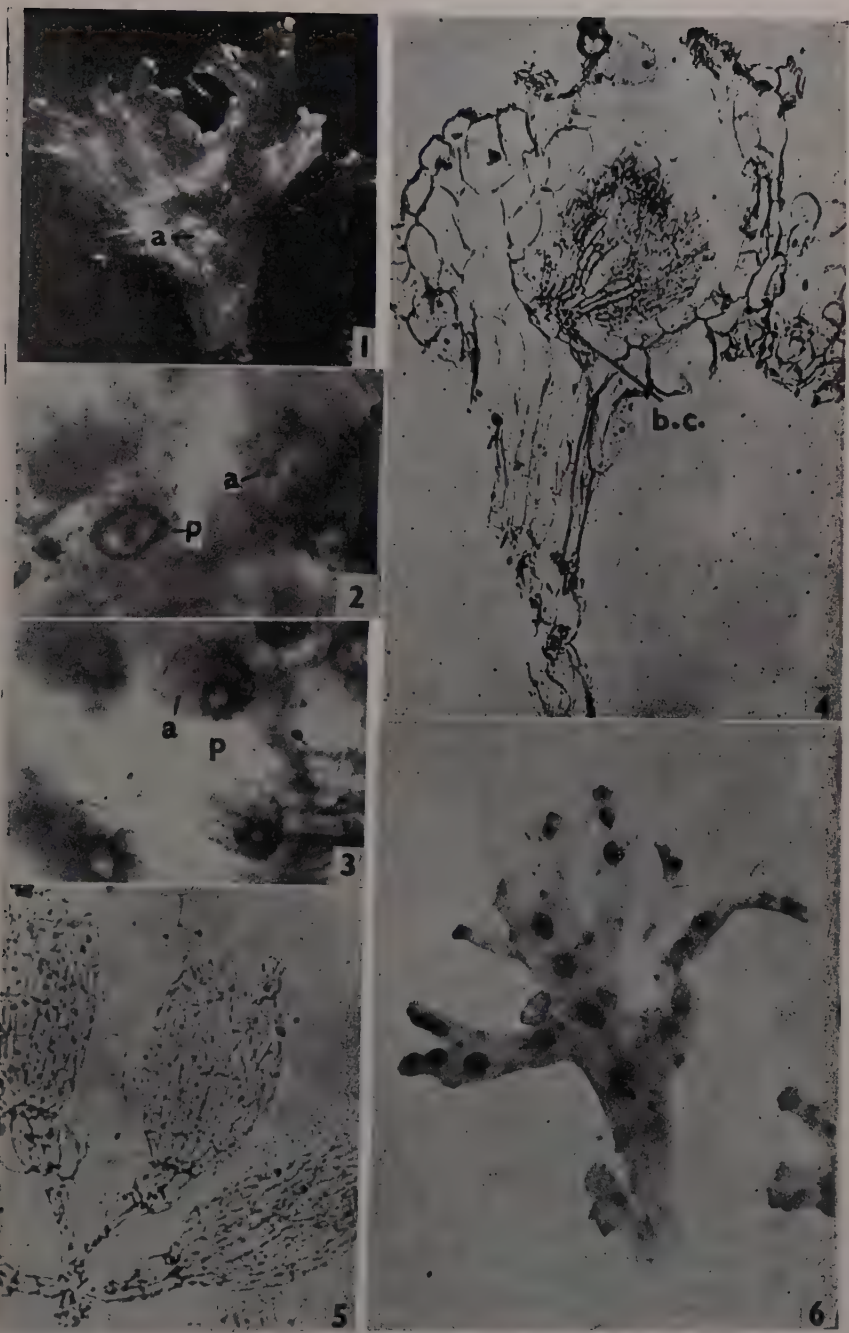
FIG. 18. *A. gemmulosus.* An elater, ×210.

FIGS. 19–21. Spores of *A. erectus.* Fig. 19. Proximal face. Fig. 20. Distal face. Fig. 21. Distal face showing width of *muri* and *luminae* and *papillae* on *muri*, All, ×500.

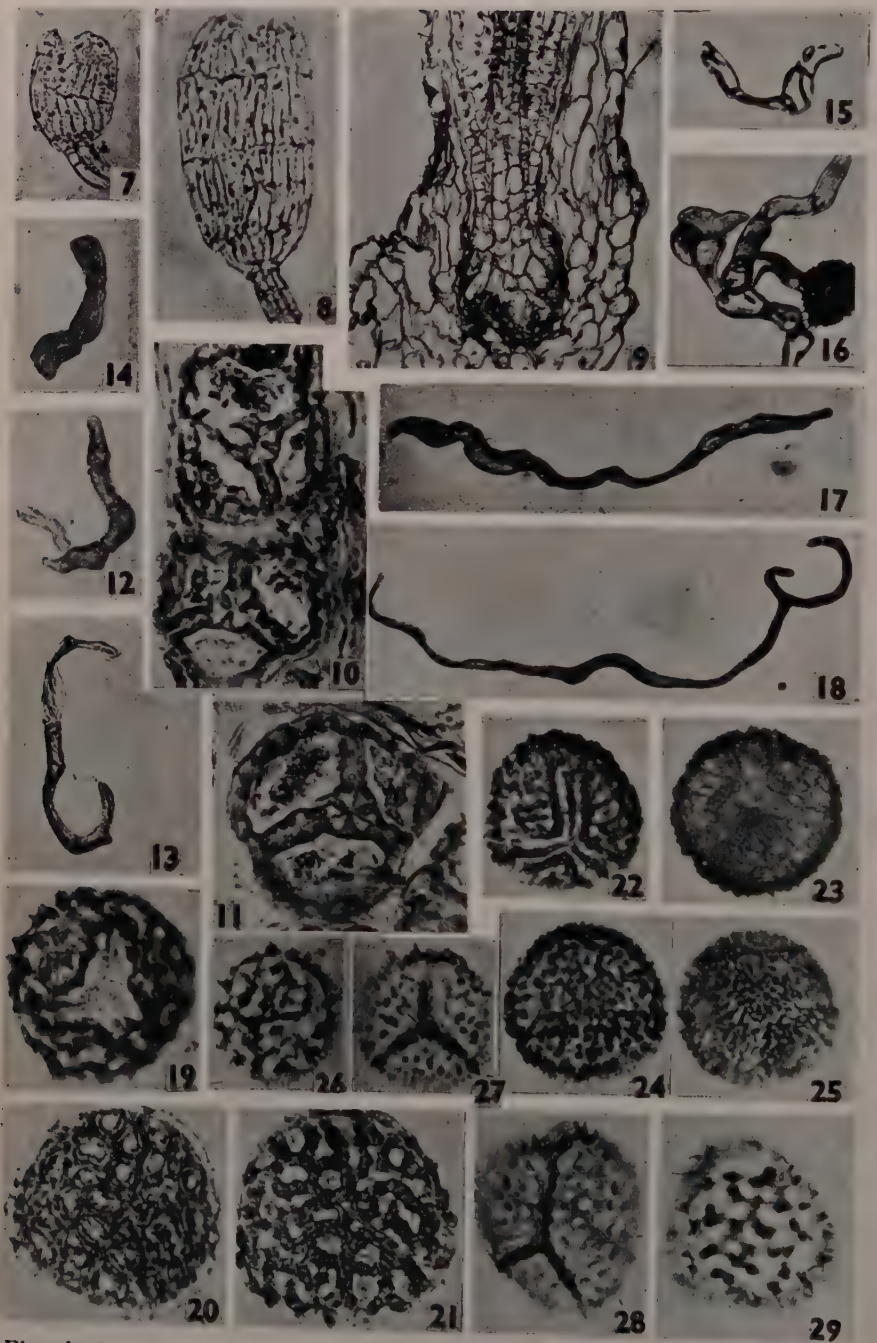
FIGS. 22–25. Spores of *A.* cf. *erectus.* Fig. 22. Proximal face. Fig. 23. Proximal face viewed from distal side. Fig. 24. Distal face deep focus. Fig. 25. Distal face in top focus showing the papillae. All, ×500.

FIGS. 26–27. *A.* cf. *subtilis.* Fig. 26. Spore in proximal view, Fig. 27. Spore in distal view. Both, ×500.

FIGS. 28–29. *A. punctatus.* Fig. 28. Spore in proximal view, Fig. 29. Spore in distal view. Both, ×500.



D. C. Bharadwaj FIGS. 1–6



D. C. Bharadwaj

FIGS. 7–29

VELAMEN IN TERRESTRIAL MONOCOTS

II.† Ontogeny and Morphology of Velamen in the Amaryllidaceae with a Discussion on the Exodermis in Amaryllidaceae and the Liliaceae

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Goebel (1922) listed a few plants of Amaryllidaceae possessing velamen. Dutt (1954) reported velamen in two species of *Crinum*. Deshpande (1955) observed velamen in a few members of Amaryllidaceae. Very recently Mulay and Deshpande (1959) published an account of the ontogeny and morphology of velamen in the Liliaceae.

Materials and Methods

Material was collected from the different botanical gardens in India and during the botanical excursions of Birla College. Roots of *Agapanthus africanus* were kindly made available to the author by Dr. Hecht of the State College of Washington. The author is thankful to Dr. Hecht for the same.

Usual procedures of dehydration, embedding and staining were followed.

Observations

Names of the plants and number of velamen layers are given in the following table.

	Name of the plant	Number of velamen layers
1.	*Agapanthus africanus* (L.) Hoff.	5–7
2.	*Alstroemeria aurantiaca* Don.	1
3.	*Amaryllis belladona* Linn.	1
4.	*Clivia miniata* Regel.	4–5
5.	*Crinum latifolium* Linn.	4–5
6.	*Crinum moorei* Hook. F.	3
7.	*Eucharis grandiflora* Planch. Lind.	1
8.	*Haemanthus coccineus* Linn.	1
9.	*Narcissus tazetta* Linn.	1
10.	*Zephyranthes tubispatha* Herb.	1

† A part of the thesis approved for Ph.D. of Rajasthan University.

The fact that the velamen is protodermal in origin has now been well established in the recent literature on velamen contributed by Mulay *et al.*

The velamen in these species varies from 1–7 layers (*see* table above). It is characterised by fine fibrillar thickenings as in *Crinum*, *Agapanthus* and *Clivia* (Text-Figs. 1 and 2) and slightly thick bands as in *Haemanthus*, *Zephyranthes* and *Amaryllis* (Text-Fig. 3). In addition to these fibrillar thickenings, the walls of the velamen cells are pitted. In *Crinum latifolium* and *Agapanthus africanus* these pits appear as cross-pits (Text-Fig. 6). Nature of these thickenings and pits is clearly revealed under crossed nicols. In longitudinal section velamen cells appear elongated along the root axis. The velamen of *Agapanthus* and *Crinum* has identical features.

Exodermis is clearly seen taking its origin from 2 or 3 prominent centrally placed cells which could be taken as periblem initials (Text Fig. 7), in such species as *Ruscus hypophyllum*, *Polygonatum oppositifolium*, *Sansevieria thyrsiflora* and many other members of the Liliaceae and in *Amaryllis belladona*, *Eucharis grandiflora*, *Zephyranthes tubispatha* of the Amaryllidaceae where the periblem is distinct. The cells flanking the periblem initials first undergo anticlinal and then periclinal divisions each cell thus forming three cells arranged one above the other (Text Fig. 7). Of these three cells the one toward the cap forms a uniseriate layer which is the exodermis; the central one undergoes periclinal divisions to form the cortex; and the cell toward plerome, undergoing only anticlinal divisions, forms a uniseriate layer which becomes the endodermis.

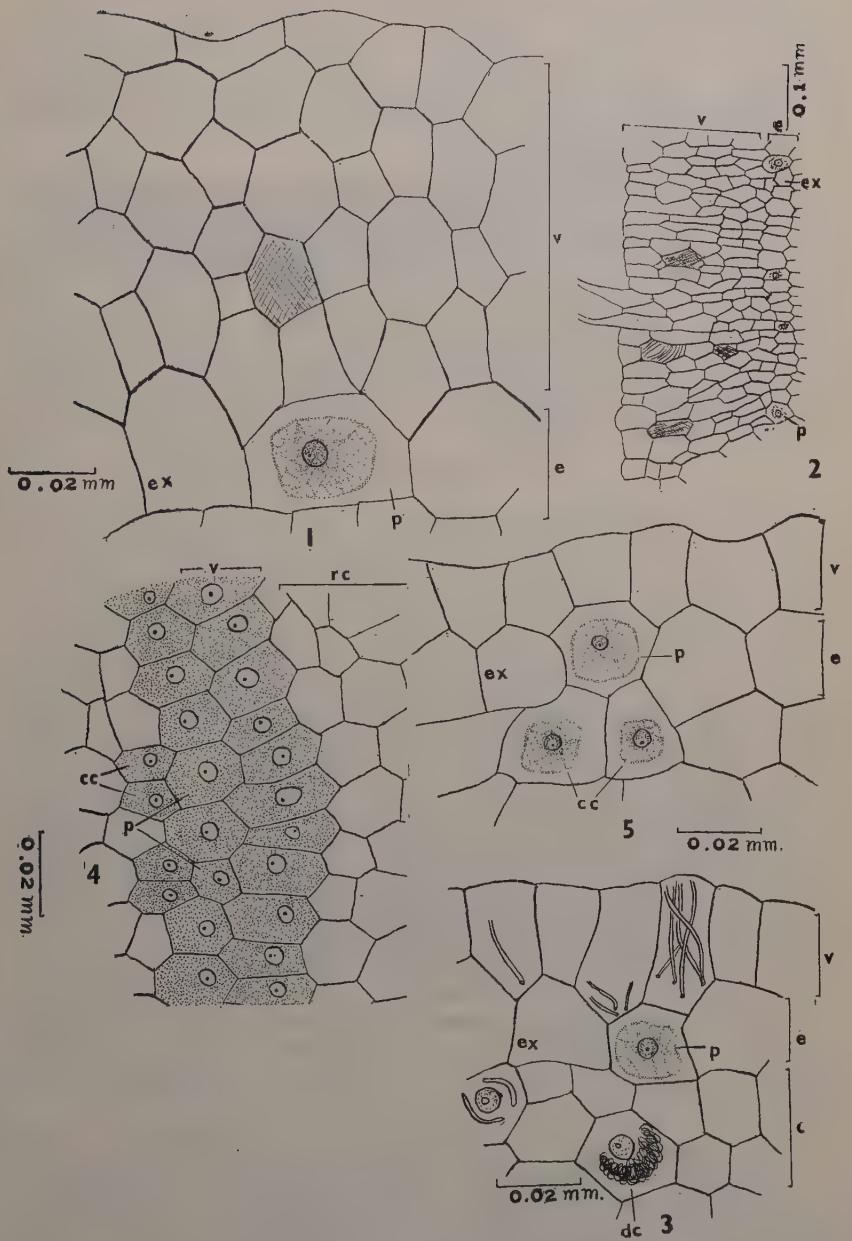
Next the origin of the exodermis may be considered in species where there are no distinct periblem initials. In such cases there is a common group of initials, for the cap, protoderm and the cortex. This condition is seen in *Tupistra clarkei*, *Hemerocallis flava* and *Aspidistra lurida* of the Liliaceae and *Agapanthus africanus*, *Haemanthus coccineus*, *Crinum latifolium* and *Clivia nobilis* of the Amaryllidaceae. In such plants the following observations suggest rather definitely that the exodermis is initiated within the cortical zone:

1. Bordering the common group of initials, there are three or four cells, more prominent than the others; of these, the cell lying nearest the plerome, forms the endodermis and the one nearest and just beneath the protoderm, the exodermis.

2. It would seem very unlikely that the exodermis would be formed from the protoderm since periclinal divisions within this tissue result in the formation of velamen (Pl. XVII, Fig. 1); furthermore, the velamen has never been known to give rise to the exodermis.

It would therefore appear that the exodermis is ontogenetically related to the cortex and not to the protoderm.

The exodermis in all the species studied, uniformly showed the following features: It is a uniseriate layer. It delimits the cortex from



TEXT-FIGS. 1–5. Portion of root of *Agapanthus africanus* in transverse section showing fibrillar thickenings. *v* = velamen; *e* = exodermis; *ex* = exodermal cell; *p* = passage cell; Fig. 2. Same of *Clivia nobilis*. Fig. 3. Portion of root of *Amaryllis belladona* in transverse section showing banded thickenings. A few cortical

cells digesting mycrorrhizal fungus. A few velamen cells also show fungal hyphae. *v* = velamen; *p* = passage cell; *dc* = digestive cell; *e* = exodermis; *ex* = exodermal cell; *c* = cortex. Fig. 4. Portion of the root of *Haemanthus coccineus* in transverse section showing early stages of the complimentary cells, passage cell and velamen. *v* = velamen ; *rc* = root cap; *p* = passage cell; *cc* = complimentary cells. Fig. 5. Portion of the root of *Haemanthus coccineus* in transverse section showing the mature region. *cc* = complimentary cells; *v* = velamen; *p* = passage cell; *ex* = exodermal cell; *e* = exodermis.

the velamen, and it consists of long exodermal cells and short passage cells (Pl. XVII, Fig. 2). The exodermal cells are the dead cells and their outer tangential walls are thick and striated. Passage cells possess protoplasmic contents and usually thin outer tangential walls. Occurrence of pits is quite common on the exodermal cell-walls. Radial walls are thick or thin and taper towards the cortex. The meristematic cell from which the passage cell is formed also gives rise to one or two complimentary cells, toward the cortex (Text-Figs. 4 and 5). These latter are living cells and have some of the features as the passage cells.

Discussion

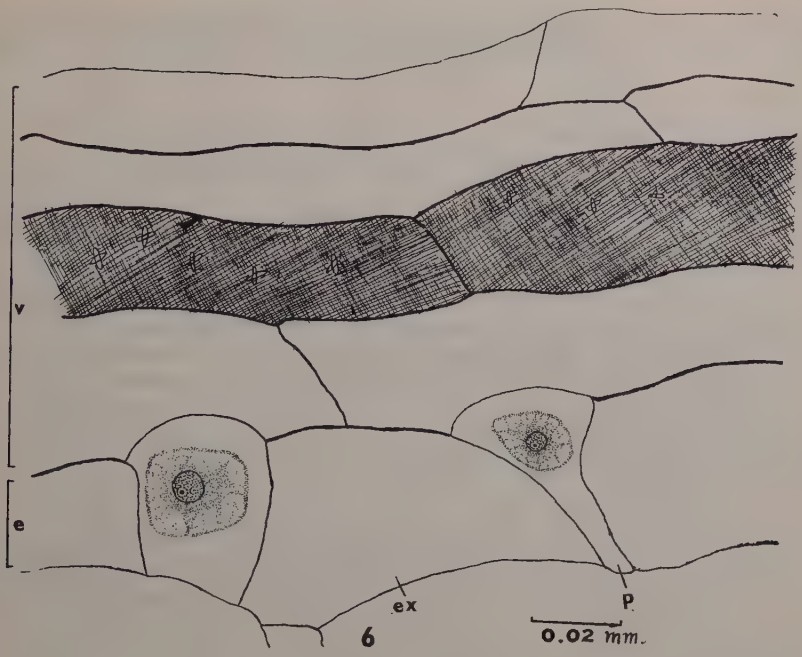
The presence of fibrillar thickenings in addition to pits on the cell walls of the velamen of the Amaryllidaceae species clearly indicate a structural advance over the velamen of the Liliaceae which is characterised by pits only (Mulay and Deshpande, 1959). The velamen of *Hemerocallis fulva* of the Liliaceae shows thickening fibrils which are to some extent similar to the fibrillar thickenings observed in *Agapanthus africanus* and *Crinum latifolium*. The latter two species have identical structure. All the above three species, *i.e.*, *Hemerocallis fulva* of the Liliaceae, *Crinum* and *Agapanthus* of the Amaryllidaceae show multiperforate plates in the metaxylem (Pl. XVII, Fig. 3).

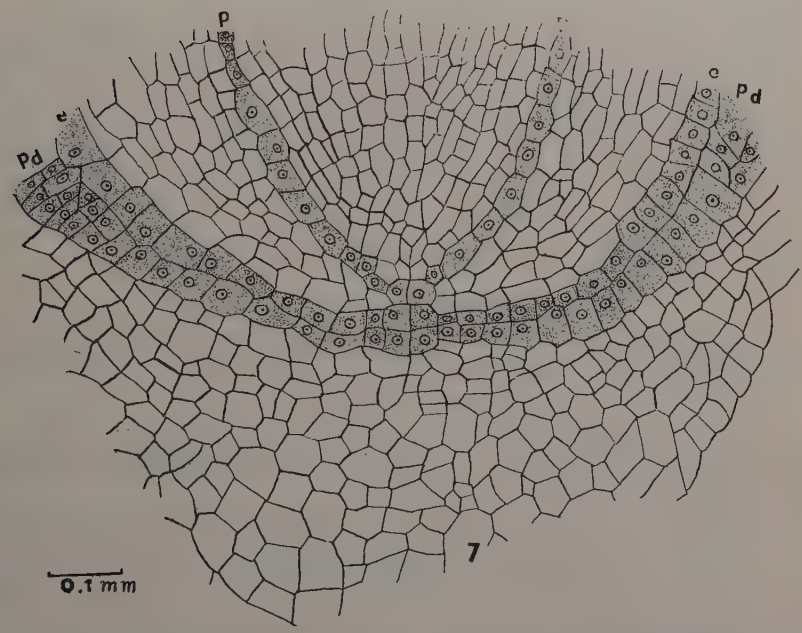
Haberlandt (1914) has stated that the outer tangential walls of the exodermis are thick but never pitted. On the other hand in the present investigation pits have been observed in the walls of the exodermal cells of the species of the Liliaceæ and the Amaryllidaceae. Occurrence of pits on the exodermal walls of the epiphytic orchids is quite common as has been demonstrated in the structure of exodermis in *Eria nana* A. Rich. and *Coelogyne barbata* Lindl. ex Griff. (Mulay *et al.*, 1958).

There is no consensus of opinion concerning the use of the terms ' exodermis ' and ' hypodermis '. Both the terms are used individually to describe several different tissues, and many a times are used synonymously.

The outermost cortical layer next to the epidermis was described as ' hypodermis ' by Jorgensen* (1878), Sachs (1882), De Bary (1884) and Sielder* (1892). Mez* (1896) designated it as a ' sclerosed layer ' and described it as a characteristic of Bromeliaceous roots. Kroemer (1903) used the term ' intercutis ' to include both exodermis and the

* Quoted by Krauss (1949).





TEXT-FIGS. 6–7. Fig. 6. Portion of the root of *Crinum latifolium* in l.s. showing cross pits and fibrillar thickenings. *v* = velamen; *p* = passage cell; *ex* = exodermal cell; *e* = exodermis. Fig. 7. Median longitudianl section of the root

apex of *Sansevieria thyrsiflora* showing the origin of exodermis. Stippled cells at the apex show apical construction. *pd* = protoderm; *e* = exodermis; *p* = pericycle.

outer cortex. Holm (1915) in describing secondary roots of *Ananas* named the outermost layer of the cortex as ' exodermis '.

The term ' hypodermis ' is still being applied to the subepidermal cortical layers in general anatomical literature. Some authors distinguish hypodermis of root under the special name ' exodermis ', while Hayward (1938), Eames and MacDaniels (1947) and Williams (1947) maintain that such an exodermis as described above is merely a type of hypodermis. Van Fleet (1950) discusses the histochemical and morphological relationship between hypodermis and endodermis, and believes the hypodermis to be the mirror image of the endodermis. According to the recent observations of Mulay *et al.* supported by the findings of the present investigations, it is the exodermis and not the hypodermis which is the counterpart and mirror image of the endodermis. All the authors who designate exodermis as hypodermis or by any other term do not seem to have taken into consideration the ontogeny of this particular tissue. According to Meyer (1940) the earlier authors studied only the morphological and functional aspect but never investigated the ontogeny and thus failed to recognise the exodermis as having a distinct origin. Her ontogenetical studies revealed that the exodermis is formed from the cortically initiated zone. Meyer's stand on this tissue has been justified by Engard (1944) who concludes from his work on orchid roots, that the term exodermis for the designation of this layer is correct. The histogenetic origin of both exodermis and the endodermis can be traced to the periblem or cortically initiated zone, its outer limiting layer forming the exodermis and inner limiting layer forming the endodermis. Engard (1944) further opines that the term ' hypodermis ', described by many earlier authors, refers to the spatial relationship with epidermis and does not show any ontogenetic relation with the cortex. This has been pointed out by Krauss (1949) also.

The following are the facts emerging out from a careful comparison of the exodermis with the endodermis, as observed in the present study and by Mulay *et al.*:

1. Both originate from the periblem or the cortical initiation zone, the exodermis forming outer limit and the endodermis the inner limit of the cortex.

2. Both form uniseriate layers, resulting from anticlinal divisions.

3. Both have cells with suberised walls with occasional individual passage cells.

4. Exodermal cells have thick outer tangential walls while endodermal cells have thick inner tangential walls. The walls of the passage cells in both the layers are thin.

5. Sometimes casparian strips which normally occur in endodermal cells may be found in exodermal cells.

6. The occurrence of passage cells in the endodermis is usually in groups of two or three. Groups of two or three passage cells have also been observed in the exodermis.

Thus, a comparative study of these two tissues shows the justification for the use of the term ' exodermis ' for the outer limiting layer of the cortex, which lies beneath the velamen.

Summary

1. The velamen in the Amaryllidaceae is characterised by fibrillar and banded thickenings and thus is more specialised than the velamen in the Liliaceae which has pits only.

2. Ontogenetically the exodermis is related to the endodermis.

3. The exodermis is a uniseriate layer delimiting cortex from the velamen.

4. A thorough comparison of the endodermis with that of exodermis indicates that the use of the term ' exodermis ' for the outer limiting layer of the cortex is justified.

Acknowledgements

I express my sincere gratitude to Prof. B. N. Mulay under whose guidance the work has been carried out. My thanks are also due to Dr. B. H. Krauss of the Hawaii University for her valuable suggestions made during the course of the investigation and for going through the manuscript. I am thankful to the Ministry of Education, Government of India, for the award of a research fellowship during the tenure of which the research was carried out.

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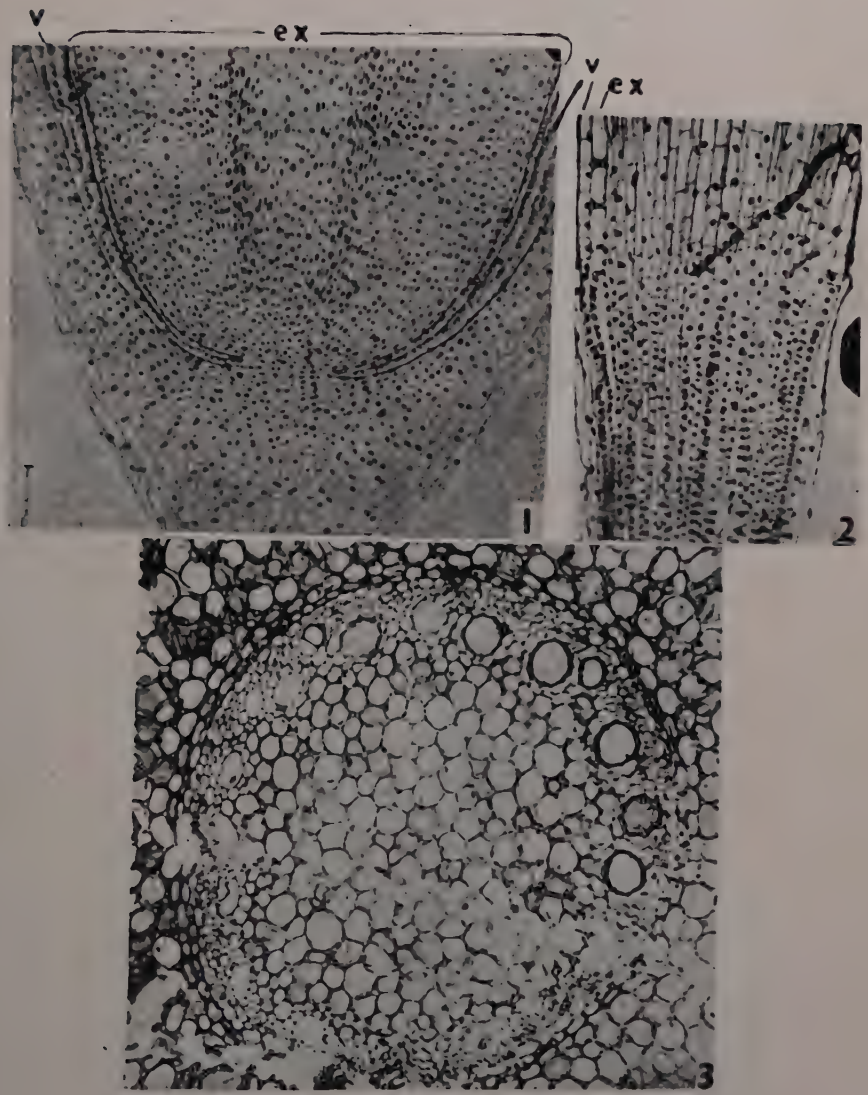
EXPLANATION OF PLATE XVII

FIG. 1. Median longitudinal section of the apical region of *Aspidistra lurida* showing the formation of exodermis and velamen. *v* = velamen; *ex* = exodermis. At the apex is a group of common initials. Retouched portion shows velamen and the exodermis, ×200.

FIG. 2. L.S. behind the root apex of *Haemanthus coccineus* showing differentiation of exodermis into long and short cells. Note that short cells have prominent nuclei and the long cells without them. External to exodermis is velamen in early stages showing nuclei, ×195.

FIG. 3. T.S. root of *Hemerocallis fulva* showing multiperforate metaxylem vessels, ×200.



B. D. Deshpande FIGS. 1–3

ECOLOGICAL NOTES ON THE VEGETATION OF KODAIKANAL IN SOUTH INDIA

By Raj Kumar Gupta

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(Received for publication on September 11, 1959)

A number of workers like Ranganathan (1938), Bor (1932), Champion (1936) and Bonnier (1905) have described the vegetation of Nilgiris ; the vegetation of Kodaikanal has not been a subject of such study. The author had the opportunity to visit Kodaikanal in the month of March and make some observations on the vegetation of this hill-station, that are given in the following pages.

Physical features and geography

Kodaikanal is a beautiful health resort situated on the southern crest of Palni plateau in Madurai district, south of the Periakulum lake between 10° 12′ and 10° 15′ North latitude and 77° 26′ and 77° 33′ East longitude. Kodaikanal proper has an elevation of about 6,900 ft. while the surrounding hills rise from 7,000 to 8,000 ft. On the north it is high and steep, on the west bounded by a ridge of considerable elevation, while from the top of the southern rim, plains are seen immediatley below.

The name ' Kodaikanal ' has been derived from the hanging woods situated on the inner side of the basin, the top of which was previously swamp but was formed into a lake by banking up the stream in 1863. The shape of the lake is like that of starfish. It is about 3 miles round as measured along the level of the road on its margins.

Climate

The temperature varies from 16·4 to 12·4° C. as against 32 to 0° C. in northern hill-stations of the country. The total rainfall is about 1,688 mm. The heaviest precipitation is during the months of August to November. (Text-Fig. 1).

Frost is of usual occurrence during the months of December to February and the clouds frequently roll up from the plains making visibility extremely dim. Mean humidity is about 74%.

Geology and soil

In the whole of the Palnis charnokites are predominant. The soil is shallow on the lower hills, at places where banana is cultivated

it is rich loam, while near Kodaikanal on the upper Palnis it is usually a black layer of peaty earth with yellow clay.

Biotic factors

Natural vegetation in the vicinity of Kodaikanal has been completely disturbed by man with plantations of *Eucalyptus*, *Pinus*, *Acacia*, etc. The sholas have been exploited for fuel and wood so much that they have practically vanished. Grazing by the cattle and fires are common in the grasslands.

Vegetation

The vegetation of Kodaikanal can be grouped under the following heads:—

1. Plantations;
2. Sholas and Grasslands;
3. Roadside Vegetation;
4. Vegetation of the Lake.

Plantations

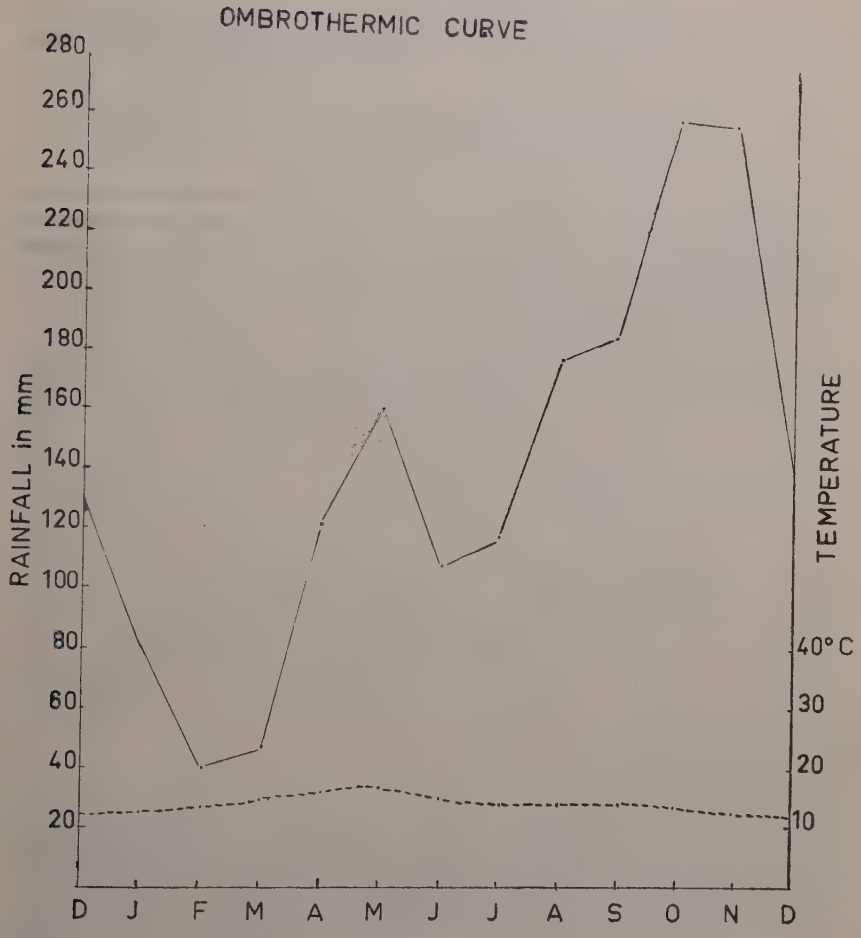
Many exotic trees have been planted near Kodaikanal and these make the spot very attractive for the tourists. The common are *Acacia melanoxylon* R.Br., *Acacia decurrens* Willd., *Acacia dealbata* Link., *Pinus roxburghii* Sarg. and *Pinus insignis* Daugal. As many as 35 species of *Eucalyptus* have been reported from Kodaikanal by Pallithanam (1957). Plantations of *Grevillea robusta* A. Cunn have been raised and are very common in the tea plantations. Below Kodaikanal at a height of 5,000 ft. there are plantations of banana, while the shade of the trees has been leased by the forest department for cardamom plantations.

Sholas and Grasslands

The sholas have been badly treated with the result that they are found only in small patches in the folds over vast stretches of grasslands.

In the sholas are present species from Ternstroemiaceæ and *Eugenia* is important with *Meliosma*, *Eurya*, *Symplocos* and several Lauraceae. The family Magnoliaceae is well represented with the species of *Michelia*; with it are found genera such as *Ilex*, *Euonymus* and *Rhododendron*. Besides that the family Ericaceae is represented by species of *Gaultheria* and *Vaccinum*.

Composition of the sholas: Eco-dominant layer.—The ecodominant layer in the sholas have been studied in the Tiger shola between altitude of 5,500 to 6,500 ft. Here the canopy is closed and the trees reach a height of about 60 to 75 ft. The main species are *Ternstroemia japonica* Thunb., *Eugenia calophyllifolia* Wight, *Eugenia arnottiana* Wight, *Eugenia montana* Wight, *Gardneria ovata* Wall., *Sideroxylon tomentosum*, *Ilex wightiana* Wall., *Meliosma wightiana* Planch, *Meliosma arnottiana*



TEXT-FIG. 1

Walp., *Elaeocarpus tuberculatus* Roxb., *Rhododendron nilagiricum* Zenk., *Euonymus crenulatus* Wall., *Glochidion neilgherrense* Wight. and *Symplocos spicata* Roxb.

Second storey consists of *Turpinia nepalensis* Wall., *Rhamnus wightii* Wt. and Arn., *Viburnum erubescens* Wall., *Psychotria congesta* Wt. and Arn., *Ligustrum roxburghii* Linn., and *Mahonia leschenaultiana* Takeda.

Besides that there are shrubs such as *Impatiens phoenicea* Bedd., *Mallotus albus* Muell., *Impatiens tomentosa* Heyne., *Gaultheria fragrantissima* Wall., *Lobelia excelsa* Lesch., *Berberis tinctoria* Lesch., and *Dodonaea viscosa* Linn.

There are some climbers such as *Rosa leschenaultiana* Wt. and Arn., *Clematis wightiana* Wall., *Elaeagnus kologa* Schlecht., *Rubus ellipticus* Sm. and *Rubus racemosus* Roxb. Ground cover is of *Galium asperifolium* Wall., *Viola canescens* Wall., *Pteridium aquilinum* Kulm., *Hypericum mysorense* Heyne, *Eupatorium glandulosum* H.B.K., *Strobilanthes kunthianus* Anders, *Osbeckia wightiana* Benth. and *Tripogon* sp. There is a heavy layer of leaf litter and mould.

Grasslands.—There are vast stretches of these grasslands in which the sholas are found interspersed in pockets. The grasses are burnt and grazed by the animals. They do not reach a good height and the main species found are *Eragrostris nigra* Nees, *Cymbopogon confeetiflorus* Stap., *Bromus asper* Murray, *Andropogon micranthus* Kunth.

Apart from these *Osbeckia wightiana* Benth., *Rhodomyrtus tomentosa* Wight., *Berberis tinctoria* Lesch. and *Rhododendron nilagiricum* Zenk. are present on the grassy slopes where the soil is little better and rainfall higher. Some plants like *Eupatorium glandulosum* H.B.K., *Ulex europaeus* Linn. and *Cytisus scoparius* Link. invade the grasslands from the roadsides. *Eupatorium glandulosum* H.B.K. is most agressive and readily takes the place of the area.

On the southern part of the plateau, where frost, fire and grazing are less, shrub association of *Rhododendron*, *Hypericum*, *Dodonaea*, *Osbeckia* and *Strobilanthes* are common. *Rhododendron nilagiricum* Zenk. is the only species that can persist in the repeatedly burnt grasslands and establishes itself either isolated or in clumps on the grassy slopes. Pure stands of *Gaultheria fragrantissima* Wall. are characteristic in this respect. Possibly they withstand frost better than any other species.

Roadside Vegetation

The vegetation on the roadsides is conspicuous, since many plants are invading the grasslands from the road. Besides *Eupatorium glandulosum* H.B.K., *Ulex europaeus* Linn. and *Cytisus scoparius* Link. there are other species such as *Hypericum mysorense* Heyne, *Gaultheria fragrantissima* Wall., *Berberis tinctoria* Lesch., *Crotalaria fysonii* Dunn., *Crotalaria scabrella* Wt. and Arn., *Alsophylla latebrosa* Wall., *Lobelia excelsa* Lesch. met with along the roadsides. Amongst the herbs mention may be made of *Erigeron mucronatum* Dc., *Viola canescens* Wall., *Lycopodium cernum* Linn., *Helichrysum bracteatum* Anders., *Vinca major* Linn., *Galium asperifolium* Wall., *Ageratum conyzoides* Linn., *Vernonia saligna* DC. and *Bupleurum distichophyllum* Wt. and Arn.

Vegetation of the Lake

In the lake not many plants have been recorded, except *Nymphaea stellata* Willd., *Carex lindleyana* Nees, *Scirpus mucronatus* Linn., *Juncus prismatocarpus* Gr. On the sides of the lake several trees have been planted. Species of *Potamogeton* Linn., *Hydrilla* Rich. and *Polygonum* Linn. are not present inside the lake.

Discussion

The ecological status of the sholas and grasslands had been a subject of discussion for a long time.

The ecological status of. the Nilgiri sholas has been discussed by Ranganathan (1938), Champion (1936) and Bor (1938).

According to Ranganathan the grasslands are the climax. Champion considers that these grasslands have come up as a result of continuous destruction of sholas and periodic firing. Bor considers that they are ' biotic climax '. More recently Shankarnarayan (1958), based on his observations on the Nilgiris, states that grasslands are the degraded stage of the sholas; the presence of some shrubs such as *Gaultheria fragrantissima* Wall., *Osbeckia wightiana* Benth., *Hypericum mysorense* Heyne and *Eupatorium glandulosum* H.B.K. are the relics of sholas. The presence of *Rhododendron* in downs also supports his contention.

Sholas

That the sholas are the climatic climax is evident by the study of the altitudinal zonation of the forest on the outer slope, and it shows that with the increase of altitude the progression is towards an evergreen climax. The shola forests have been found to be a stable plant community in equilibrium with the climatic and edaphic factors of the folds in the plateau. There is no difference of opinion on this point.

Grasslands

There is a school of thought that the scattered shrubs of *Rhododendron* are the vestiges of the destroyed sholas. The *Rhododendrons* are strong light demanders and do not regenerate freely in the sholas, but do so in the open, in spite of adverse conditions. This would indicate that they are one of the pioneer tree species in the progression towards the shola formation and tending to disappear with full development of the sholas. The scattered trees found may be regarded as one of the primary stages arrested from further progression by factors inimical to the shola formation.

Ranganathan (1938) is of the opinion that these grasslands are the climatic climax over greater part of the Nilgiris. According to him these grasslands exist since times immemorial ; the population of Todas does not practise agriculture, nor clear forests. Grazing and burning of the forest vegetation could reduce the forest climax into a poor scrub stage, as may be seen in other parts of the locality. In the Nilgiris these grasslands owe their origin to frost as the climatic factor.

But the idea of Ranganathan cannot be accepted ; it has led to the recognition of two fundamentally different climatic regions.

Champion, however, is of the view that grasslands are nowhere climatic climax formation in Nilgiris. Periodic firing of grasslands by grazers has caused the present distribution of grasslands and sholas.

His theory does not explain why a more or less stable climax of grassland should be established in an area with good rainfall by periodic firing alone; when such treatment of old clearings has not led to such extensive grassy stretches elsewhere, but is always associated with a degraded secondary shrub.

Bor is of the opinion that once we admit the existence of grazing and burning in the area, we cannot apply the term 'climatic climax' and so these grasslands should be considered as biotic climax.

Recently Shankarnarayan (1958) has written that the Nilgiri sholas are a subclimax governed by a set of biotic factors which do not admit easy passage to the final climax.

Since it is universally accepted even by Ranganathan that sholas are the climatic climax of the region, there cannot be two climaxes in a locality within the same climate and the burning and grazing can reduce the forests almost to scrub, and such formation of grassland over extensive areas in view of the rainfall cannot stabilise permanently. According to some of these grasslands cannot be considered as subclimax, which is prevented from progressing to the climatic climax only by biotic factors; because in a subclimax, when once the retarding force is checked, there is a tendency to progress towards the climax, but in these grasslands even in areas where grazing and burning is not so prevalent, these grassy patches seem to be held intact for a long time. In these areas certain climatic factors such as frost, lack of sufficient soil moisture and drainage, except in the folds of the hills, play an important part which prevent sholas over a vast area and stabilise the grasslands. So the grasslands should be considered as a preclimax which is governed by a set of natural factors which do not admit easy passage to the final climax. Where the grasslands are associated with stunted shrubs and other species, as at the edge of the sholas, they should be considered as a subclimax prevented from reaching the climax by certain set of biotic factors.

Where the conditions are favourable in the sheltered folds of the hills (prevented from exposure of wind, sufficient soil moisture, and good drainage) grass is succeeded by herbaceous vegetation and shrubby species as *Dodonaea viscosa* Linn., *Hypericum mysorense* Heyne, etc., at the first instance and finally by *Rhodomyrtus*, *Berberis*, etc., and in the shelter of these natural sholas develop. Afforestation of the grasslands with shola species tried from 1934 indicate that shola species can grow and regenerate under the forest cover provided by *Cytisus scoparius*. Further forest department has successfully grown a shola forest in the Nilgiris, which has attained a good growth by mere protection from grazing and fire, and wherever the grasslands are protected from biotic influence the shola species come up.

This leads the author to believe that the grasslands are a *subclimax* which do not admit easy passage to the final climax on account of certain biotic and other factors.

Summary

1. Ecological notes on the vegetation of Kodaikanal are presented in the paper.

2. Location and topography of the area under consideration have been described.

3. Environmental factors such as climate, geology, soil and biota have been given.

4. The vegetation has been grouped under the 4 categories : (1) Plantations, (2) Sholas and grasslands, (3) Roadside Vegetation and (4) Vegetation of the lake.

5. Views on the status of shola grassland formation have been discussed.

Acknowledgements

I am deeply indebted to Prof. P. Legris, Director, French Institute, Pondicherry, for his valuable suggestions and excellent facilities provided during the tours; to Rev. Fr. Santapau, for his criticism and to Dr. G. S. Puri for his kind help and suggestions during the course of the work.

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* Original not seen.

SOME OBSERVATIONS ON THE CYTOLOGY AND APOGAMY OF HIMALAYAN *DRYOPTERIS PALEACEA* (DON) HAND-MAZZ.[1]

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Considerable cytological work has been done on the genus *Dryopteris* Adanson in Europe and North America (Manton, 1950; Wagner, 1955; Walker, 1955) but so far nothing has been known about the Himalayan species of this genus. Out of the 20 species studied so far by the writer, at least 5–7 including the present one have been lumped as varieties of *D. filix-mas* (*sensu-lato*) by Clarke (1880) and Beddome (1892) because of considerable uniformity in their morphology and habit. Ching (1938) gave specific rank to almost all these varieties on morphological ground and his taxonomic treatment of the Himalayan taxa seems broadly justified as evidenced by the cytological studies of the present writer.[2]

Unlike most other Himalayan species, *D. paleacea* has a very wide distributional range (*cf.* Ching, *l.c.*; Nordhagen, 1947) and has been treated by some recent workers (Rothmaler, 1943; Dopp, 1954) as a synonym of the European *D. borreri* Newm. which is a species complex with several polyploid, obligately apogamous races (Knaben, 1948; Manton, 1950). The first cytological report on the species is by Manton and Sledge (1954) who found triploids with n and $2n = 3x = 123$ in Ceylon. The present investigation deals with cytology and some important aspects of the apogamous mode of reproduction. On the basis of available cytogenetical data, the suppression of one of the sexes (usually the archegonia) in obligate apogamous ferns is discussed.

Observations

The species grows commonly in Darjeeling between 7,000–9,000 ft. alt. on the forest floor in relatively moist and shaded environs. The rhizome is stout with a tendency to grow erect and in certain protected localities it may attain a length of 1–1½ ft. Like many other species of the area, during each growing season the leaves grow in a rosette around the shoot apex, thus presenting a basket-like habit (Plate XVIII, Fig. 1).

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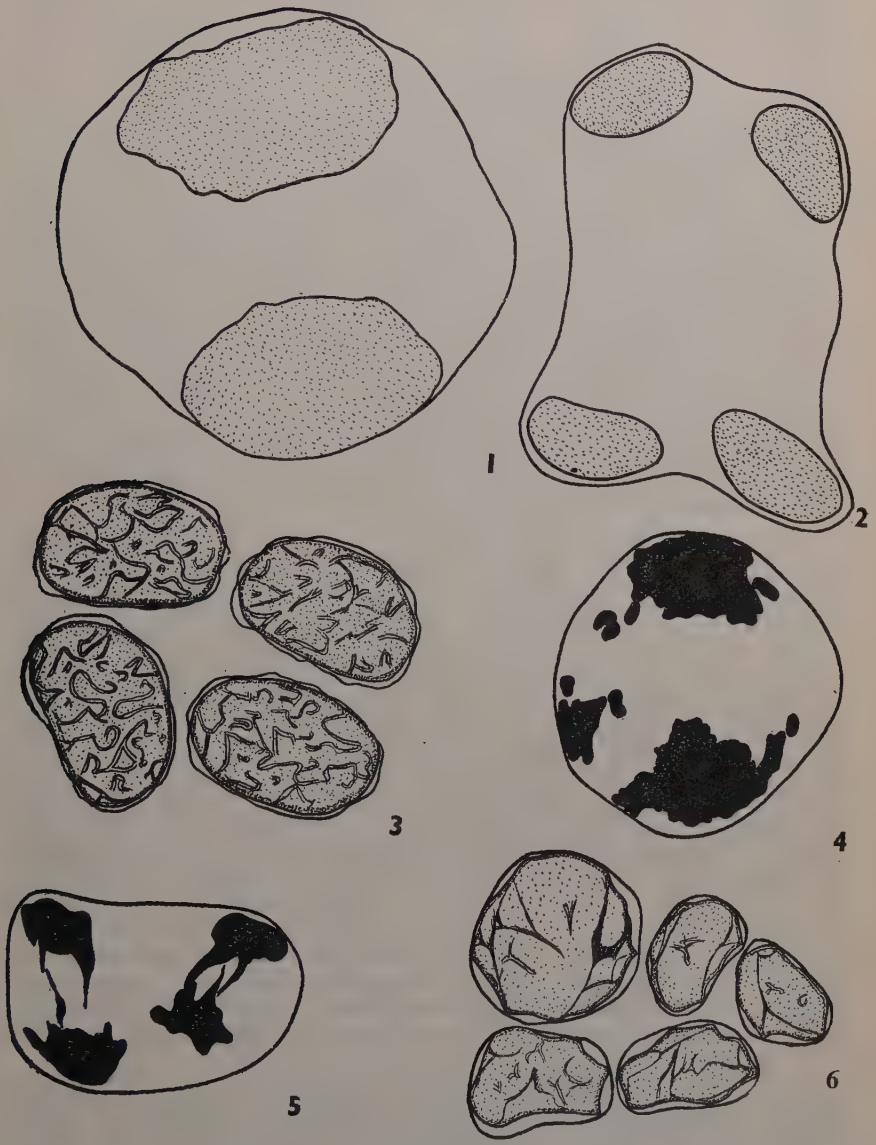
[1] According to Alston (1957) the name *D. paleacea* for the Asiatic plant is invalid and should be replaced by *D. wallichiana* (Spreng.) Hyld.

[2] This work is being completed and will be communicated shortly.



TEXT-FIGS. 1–6. Figs. 1–2. Spore-mother-cells from eight-celled sporangia showing normal diad and tetrad formation, ×1,300. Fig. 3. Normal spores from an eight-celled sporangium, ×400. Figs. 4–5. Spore-mother-cells from sixteen-celled sporangium showing abnormal telophase—I and II, ×1,300. Fig. 6. Spores from a sixteen-celled sporangium, ×400.

The species has been primarily sampled from several localities along the Indo-Nepalese border, but material from Northern Sikkim has also been studied with identical results. Acetocarmine squash preparations of a large number of spore-mother-cells in the eight-celled sporangia, show 82 'auto-bivalents' (*sensu* Hakansson and Levan, 1957) at diakinesis (Plate XVIII, Fig. 2). Thus, the present species differs from the Ceylonese material in being a diploid based on 41 as the gametic number. The formation of diads and tetrads is perfectly normal (Text-Figs. 1 and 2) and 32 spores are formed in a sporangium as is usual of apogamous ferns (Text-Fig. 3). The sixteen-celled sporangia, whose production is not more than 3–4% of the total output, were also studied. The spore-mother-cells in this case follow an irregular course of meiosis. Owing to the rarity of such spore-mother-cells the exact numerical data of the paired and unpaired chromosomes could not be obtained. However, in somewhat clearer preparations, 37–39 paired chromosomes were counted at diakinesis. The number of unpaired chromosomes varied 4–8 which are not included in the resulting nuclei (Text-Fig. 4). At the end of anaphase-II chromatin bridges are seen (Text-Fig. 5) and as a result of these abnormalities, shrivelled and apparently non-viable spores are formed (Text-Fig. 6).

The gametophytes of the species were raised in September 1957, and were kept under observation till April 1958, after which they perished owing to summer heat of the plains. The earlier stages were similar to those seen in the other species of the genus studied by the writer. Most of the prothalli became heart-shaped in about 2½ months while the rest remained filamentous or ameristic and developed antheridia copiously at later stage. A critical study of heart-shaped prothalli at this stage showed a conspicuous region of smaller cells with denser cytoplasm situated 4–5 cells posterior to the notch. These actively dividing cells in the various planes, formed young sporophytes which are protected in the initial stages by multicellular, brownish ramenta and also capitate glands similar to those on the gametophyte. The presence of these scales on the young sporophyte appears to be a uniform character in other apogamous species as well. This serves as a strong indication of the apogamous origin of the sporophyte because the first-formed leaf in sexual species usually lacks such scales which appear on the subsequent ones. Some of the prothalli showed a few tracheids similar to those observed earlier by Mehra (1938) in other apogamous ferns. Archegonia were not observed on the prothalli in these cultures.

It is of some interest to mention that the prothalli bearing apogamous buds usually lack a normal well-developed cushion and remain single or double-layered throughout the central region. In strong contrast to this, in many sexual species of this genus, several layered thick cushion has been observed by the writer. It is reasonable to assume that the cells, whose meristematic activity leads to the formation of a cushion in sexual species, start functioning through some stimulus of unknown nature as initials of the sporophyte in an obligate apogamous species. This is borne out by the fact that the ameristic prothalli usually never bear apogamous buds. It is pertinent to point

out that this aspect of apogamy seems to have not been dealt with by previous workers and an experimental study is likely to throw light on the mechanism involved in suppressing the formation of archegonia in obligate apogamous species.

Discussion

The role of cytogenetical studies in ferns has been most significant in so far as the problem of evolution of the European *D. filix-mas* complex is concerned. It is now known that there are three taxonomic species comprising the 'filix-mas complex' (Manton, *l.c.*). One of these is *D. abbreviata* (Lam and DC) Newm. with $n = 41$ and *D. filix-mas sensu-stricto*. emend is tetraploid sexual. Morphologically different from these two is *D. borreri* Newm. which is an obligate apomict with cytological races ranging from diploid to pentaploid. The present study reveals that *D. filix-mas* (*sensu-stricto*) does not occur in the Himalayan region so far visited. The present species is comparable to *D. borreri* Newm. in being an obligate apomict. Primarily in view of this character and perhaps morphological resemblance as well, Dopp (*l.c.*) and Rothmaler have favoured their amalgamation. On the other hand, Ching (*l.c.*) firmly believed in their treatment as separate species. Furthermore, Alston (*l.c.*) has recently stated that the Asiatic plants possess blackish scales and are therefore distinct from the American plants with reddish brown scales. A representative collection from Darjeeling-Himalayas shows that the colour of the scales cannot be used as a safe criterion for their segregation, since the Himalayan plants usually possess rusty-brown scales. Besides, there may be present blackish brown scales as well either on the same or on different fronds but with no other morphological difference. The same variation of colour has been noted earlier by Ching (*l.c.*) in specimens from other parts of Asia. Thus it is evident that the entire problem of its taxonomic status demands a critical cytomorphological study from other parts of its range. The writer is at present inclined to treat this taxon tentatively under *D. borreri* apogamous complex as done by Dopp and Rothmaler. Furthermore, Manton (personal communication) has also put forward her views in favour of this suggestion as she has studied a large part of this complex.

The mode of chromosome pairing in the sixteen-celled sporangia in an apogamous species is of special interest (Manton, *l.c.*; Tryon and Britton, 1957), because it represents their true genomic homologies, which in turn is likely to throw light on the evolution of the species. Two tentative conclusions with regard to the origin of the present species emerge from meiotic studies in the sixteen-celled sporangia. An unusually high preponderance of paired chromosomes indicates that it may be an intervarietal cross having a few non-homologous chromosomes which fail to pair and ultimately not included in the tetrad nuclei. However, no sexual plants with close morphology have been discovered so far. Its morphological resemblance, to some extent, with *Ctenitis apiciflora* (Wall.) Ching (*cf.* Ching, *l.c.*) perhaps may not imply any whole-scale genetic relationship but strongly suggests that the two genera,

namely, *Dryopteris* Adanson and *Ctenitis* C. Chr. are very close and some of their species are in a parallel course of evolution. The second probability is that it may have originated from a formerly sexual species through chromosomal aberrations which result in the formation of bridges and overall disturbed meiosis. Such cytological changes can well be suspected particularly because, in view of the very wide geographical distribution, the taxon appears to be fairly antique. It is quite likely, therefore, that in this species apogamy might have arisen through a process other than hybridization.

As pointed out earlier, the gametophytes of the present species are totally devoid of archegonia and produce functional sperms in abundance. Similar observations have been recorded earlier in other obligate apogamous ferns (Manton, *l.c.*; Tryon and Britton, 1957). It may reasonably be concluded that in true apomictic ferns pre-meiotic doubling of chromosomes which ensues some kind of genetical unbalance, manifests itself in suppressing the formation of archegonia in the gametophyte.

Summary

The cytological studies on the Himalayan *D. paleacea* shows that it is a diploid, obligate apomict with n and $2n = 82$. The eight-celled sporangia produce normal 32 spores. In sixteen-celled sporangia, majority of the chromosomes are involved in bivalent formation and the lagging univalents are not included in the tetrad nuclei. Variable number of inviable spores are formed in each such sporangium. The origin of the species has been discussed on the basis of chromosome pairing in the sixteen-celled sporangia.

The gametophytes develop apogamous buds at a rather early stage thus eliminating the normal cushion in the central region. On the basis of present study as well as available data on other apogamous ferns, it is suggested that the absence of archegonia is a genetical phenomenon succeeding the pre-meiotic doubling of chromosomes.

The question of its taxonomic relationship with *D. borreri* cannot be answered at this stage. However, in view of the present data a close morphological and genetical relationship is evident. This aspect needs much more critical study on a comparative basis from other parts of its range. Further work in other parts of Himalayas is in progress.

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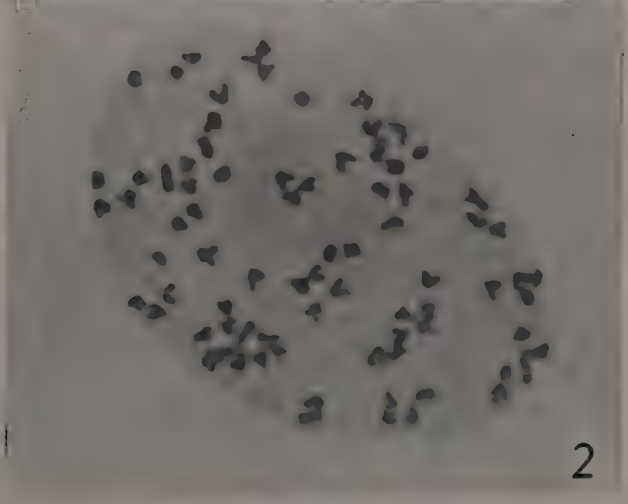

D. S. Loyal FIGS. 1-2

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Explanation of Plate XVIII

Fig. 1. Habit of the plant, note almost erect posture of the rhizome.

Fig. 2. Spore-mother-cell from an eight-celled sporangium showing 82 autobivalents at diakinesis, ×1,500.

ECOLOGICAL STUDIES ON THE FUCALES

I. *Pelvetia canaliculata* Dcne. *et* Thur.*

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Introduction

Considerable work has been done on the taxonomy, distribution and synecology of marine algae. There are also many accounts on the recolonization of artificially denuded areas, rate of algal growth, yield and so on. Yet, the contributions on individual species are very few. A perusal of the literature revealed that our knowledge especially of the algae of the upper littoral belt was far from complete. Investigations were, therefore, undertaken on two common Fucales occurring in U.K. coasts, *Pelvetia canaliculata* Dcne. *et* Thur., and *Fucus spiralis* L., at the kind suggestion of Dr. Margery Knight, with particular reference to the recolonization, by the two species, of cleared areas on the shore and their yield, as also their rate of growth and reproductive cycle. The present part deals with an ecological study of *Pelvetia canaliculata* based on a study over a period of two years.

The Stations and Environmental Factors†

The investigation was carried out at two stations in the south-west coast of the Isle of Man, at Port Erin and Poyll Vaaish, the latter a shore about three miles away from Port St. Mary. At Port Erin, the experimental area is located just opposite the Biological Station and the area can be described as a " boulder beach " sheltered by the damaged breakwater. At Poyll Vaaish, the beach is of boulders and sloping terraces which are very much fissured. At both the stations the usual belts of vegetation of the Fucaceae are found, beginning from the top the order being *Pelvetia cannaliculata*, *Fucus spiralis*, *Ascophyllum nodosum*—*Fucus vesiculosus*, and *Fucus serratus*. The width of the belts depend on the slope of the surface and the density of the vegetation varies inversely with the exposure of the area to the prevailing winds, this

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* Edited for publication from part of the *Thesis* accepted for the Degree of Doctor of Philosophy of the University of Liverpool, U.K. A copy of the *Thesis* is deposited in the Harold Cohen Library of the University. Paper read before the Challenger Society (Subrahmanyan, 1948).

† The particulars under this head relating to the tides, rainfall and hours of sunshine were kindly supplied by Mr. Brown of the Harbour Commissioner's Office, Douglas, I. O. M. and those relating to salinity, temperature and prevailing wind were obtained from the records kept in the Biological Station, Port Erin.

being particularly noticeable in the case of *Pelvetia* and *Ascophyllum;* the former is completely absent in places where the fury of the waves is strongly felt. The prevailing winds are westerly and the vegetation of *Pelvetia* is very prominent in places sheltered from this wind as at Poyll Vaaish.

Observations showed that neap tides on calm days do not reach the *Pelvetia*-zone and at best just touch the fringe of it only, but, spring tides completely cover the zone. This would mean that *Pelvetia* extends from H.W.N.T. to H.W.S.T. *Fucus spiralis* comes next below *Pelvetia.* According to Darbishire (1902, p. 30) *Pelvetia* extends from 12–17 feet above 0 (L.W.O.S.T.) and from the sequence of the discussion in his account, it is possible that he refers to Port Erin.

The variation in salinity, as gathered from the readings kept at Port Erin, is very little. It varies from 32·9–33·5‰, the highest value coinciding with the period of higher temperature, increased sunshine and lesser rainfall.

The mean temperature (taking the maximum recorded each day) of the air varies between 4·33° C. and 17·83° C. and that of the sea 9·77° C. and 13·83° C. in the course of a year. The temperature of air is lowest in January and highest in July; for the sea, the lowest value is reached at the end of February and highest in August. From the middle of September to the end of February the mean temperature of the sea is higher than that of air.

During the year, the mean number of hours of sunshine per month varies from 43 hours in December to 231 hours in June.

As the *Pelvetia*-zone is almost left uncovered by the tides for days on end during neap tides, precipitation of moisture by rain may have an important effect on the existence of the vegetation in that zone. The mean annual rainfall for the Isle of Man is 44·6 inches; it is least from April to August and increases from September to January. It may be inferred that *Pelvetia* is able to withstand great variations in the concentration of the medium apart from drought, for, if there be rainfall when the tide is out, it is drenched by the rain-water with no adverse effect.

Methods

A number of patches of the vegetation in the respective zones was cleared of all the plants at different times in the course of a year and the area thus cleared was marked off by quick drying enamel paint. Observations were made on the areas at intervals and the new growth of the plants on them recorded. It was sought to study in this manner the order of colonization of the areas, the rate of growth of the plants, their number and quantity. The " normal population " obtained originally after clearing the area was also carefully examined after being brought to the laboratory. To obtain an idea of the composition of the normal population, the plants were sorted out into different size

groups, size being arbitrarily chosen as there was no means of assessing the age of plants at the beginning of the experiments; and, the number of plants in each group, the weight of each group, the state of the plants —whether reproducing or not—were all recorded.

To obtain the rate of growth, individual plants of all sizes were also observed at intervals. For *Pelvetia* a ring of quick drying paint was applied around the base and the plants numbered. The same method was adopted for young *Fucus* plants, but, the older ones were marked by slipping on to the stipe a numbered celluloid disc in such a way that the plant was not injured.

To study the reproductive cycle, a definite number of plants were critically examined every month. The numbers of sterile tips and reproductive tips (receptacular ones) of the fronds were noted and hand-sections were made to determine the state of the reproductive bodies inside the conceptacles. Some definite stages were recognized from the beginning of the reproductive cycle up to the end of the season and the numbers of receptacles in each such stage was also recorded.

Recolonization

Five patches were cleared in the *Pelvetia-zone* when the tides were favourable, between November 1945 and June 1946, one at Port Erin and the rest at Poyll Vaaish, two of $\frac{1}{2}$ M^2 area and three of 1 M^2 area. The areas were cleared completely of all the plants and the surface scraped bare. It is possible that some minute plants were left on the areas particularly in the crevices.

These cleared areas were periodically visited and the number of plants in the various size-groups recorded, and also other interesting points such as presence of other algae and predatory animals, etc. Owing to limitations of space, all data collected during the several visits cannot be presented here. Mention may, however, be made about the presence of *Calothrix* sp. growing epiphytically on the basal portions of young *Pelvetia* in Area I and of the presence of predators like *Littorina* sp. and *Patella* sp. which take a toll of the juvenile populations on several of the areas.

As the main purpose of the investigations was to assess the rate of growth and production of matter by *Pelvetia*, the relevant data have been abridged and given in Tables I and II.

It may be seen from Table II that the rate of growth in length varies from 1·8–4·8 cm. per year, the average being 3·18 cm./year.

The yield of *Pelvetia* on re-clearance was found to be 373 gm./year/M^2.

Judging by the maximum size attained in an area and the average rate of growth observed, it would appear that a period of over 5 years must elapse before the original population is restored in an area.

TABLE I

Normal population, Pelvetia canaliculata *zone*

Area		Date of clearance	*Pelvetia*-size groups in cm.							*Fucus spiralis*	
No.	Extent in sq. meters		Less than 3	3–6	6–9	9–12	12–15	15–18	Weight of total yield in gm.	No. of plants and size	Weight of yield
I	1	11–11—1945	500	421	276	78	51	20	2977	..	..
II	½	27–11—1945	749	237	201	110	50	..	1492	47 0–15 cm.	67
III	1	5—1—1946	400	397	143	227	70	..	1773	44 0–15 cm.	41
IV	½	2—4—1946	2197	415	..	165	134	50	2663	32 5 cm.	1·5
V	1	22—4—1946	1361	324	665	273	105	..	3085	..	..

TABLE II

Repopulation, Pelvetia canaliculata *zone*

Area No.	Extent in sq. meters	Date of final observation	*Pelvetia*		Maximum size reached in cm.	Period n months	Rate per year in cm.	Yield on re-clearance	Yield per year *Pelvatia*	Remarks
			⩽3cm.	3–6 cm.						
I	1	3—4—1947	14462	179	6	17	4·5	528	373	Some *Fucus spiralis* at times, at the bottom of the square. Disappeared and reappeared later, new growth. On re-clearing, *Fucus* yielded 8 gm. and *Enteromorpha* 3 gm.
II	½	1–10—1947	104	3	6	22	2·7	..	..	Some *Fucus* specially at the bottom
III	1	30—9—1947	14	..	3	20	1·8	..	..	do
IV	½	30—9—1947	415	..	3	17	2·1	..	..	..
V	1	30—9—1947	461	18	6	15	4·8	..	..	..

Position and Nature of Surface:

I. An almost vertical face of rock facing south in a sheltered situation with numerous depressions. Poyll Vaaish.

II. Two sides of a boulder, part of it vertical; rest horizontal with few crevices. Sheltered by larger boulders and breakwater. Port Erin.

III. Exposed area; horizontal, slightly sloping rough surface with numerous depressions. Poyll Vaaish.

IV. Flat strip of rock with several clefts and tiny depressions sheltered by a lightly rising rocky terrace towards sea. Poyll Vaaish.

V. Edge of a ridge of rocky shore, horizontal, surface full of depressions affording shelter to groups of plants. Poyll Vaaish.

The average yield of *Pelvetia* from a metre-square area is 2998 gm., based on first clearance of the five areas. It may be noted that Area I yielded 2,977 gm. on first clearance, nearer to the average. The yield, based on an area (Area I) re-cleared after an interval of 17 months was found to be 380 gm. per annum. This can at best be only a minimal estimate as the population concerned consisted of juvenile plants.

Marked Plants

A large number of plants were marked to observe their growth, but only a few remained for some length of time to yield some data. The survivors were all small plants. The data recorded from this source are given in Table III. The plants were in a very sheltered situation.

From the data shown in Table III, it will be seen, that plant No. 5 has a very low value for growth rate. This may be exceptional. If we omit this, we get a value of nearly 3·9 cm. a year which is very near to the average value obtained from repopulation studies, *viz.*, 3·18 cm. (average from all Areas I–V) in a year. The increase in the number of tips indicates the increase in the bulk of the individuals.

Reproductive Cycle

Period and Stages

Pelvetia has only one reproductive season in a year. In the Isle of Man, the season begins in January and lasts till the following October, or November. The peak in the period as witnessed by the release of the reproductive bodies is attained in August and lasts up to the early part of September.

The earliest signs of reproduction were observed in January. This becomes evident when the apical pit tends to be obliterated and a slight swelling of the tip of the frond takes place (Pl. XIX, Fig. 6, stage A). In a few of the plants obtained from Millport in the middle of December, 1946, the early stages resembling the stage described above were noticeable. An extremely small percentage of the tips of the fronds of the alga growing at Port Erin showed the stage in the last week of December.

Soon in such swollen tips conceptacles become distinguishable. Subsequently the conceptacles show the oogonial initials followed later by the antheridial initials (Pl. XIX, Figs. 1 and 7, stage B). By the end of June oogonia and antheridia become fairly common, the eggs and antherozoids being differentiated to a certain extent (Pl. XIX, Fig. 1, stage C). July may be said to be the ripening period for the contents of the conceptacles, for dehiscence of reproductive bodies begins in August (Pl. XIX, Figs. 3 and 4, stage D). Dehiscence soon reache sthe peak period and with its waning in September, the receptacles turn yellow (Pl. XIX, Figs. 2 and 3, stage E), deepen into orange and degenerate (Pl. XIX, Fig. 2, stage F).

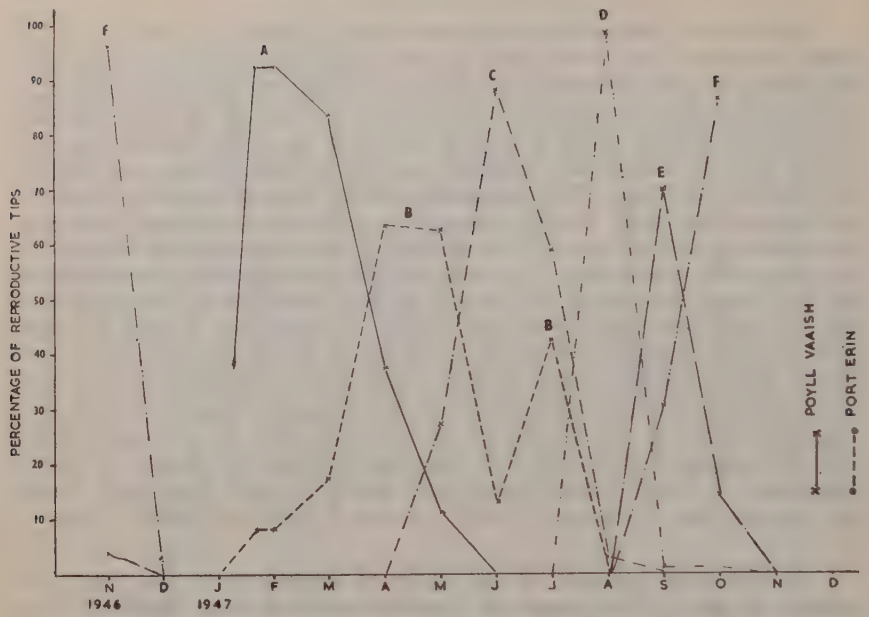
TABLE III

Pelvetia canaliculata—*Marked plants*

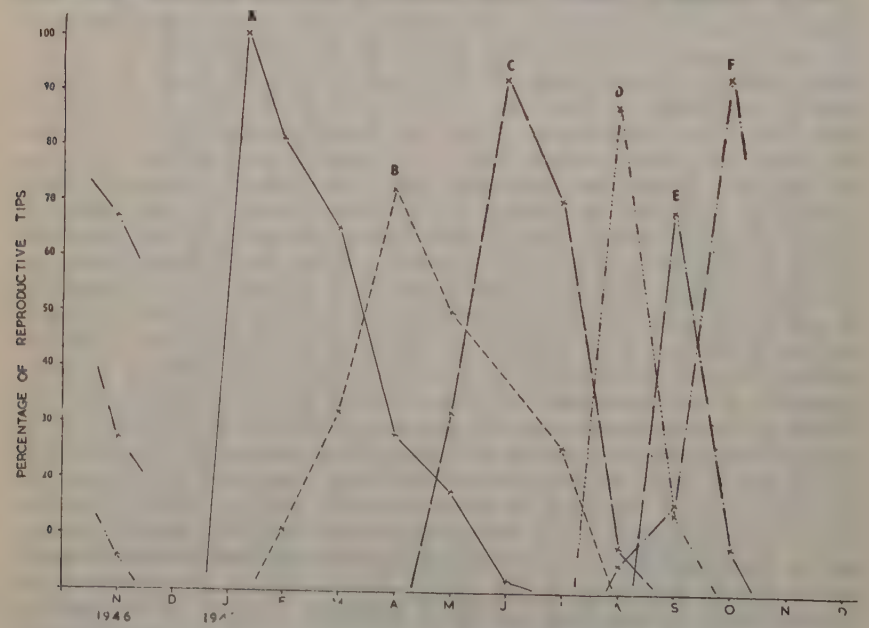
Plant No.	Dates of observation					Rate of growth	Increase in tips	
	12—4—1946	6—8—1946	7—11—1946	2—1—1947	30—3—1947		Tips	Period (months)
1 Length in cm. ..	5	6·4	Plant missing	..	..	1·4 cm. in nearly 4 months. 4·2 cm. in one year	81	4
No. of tips ..	111	192	..	..	..			
3 Length in cm. ..	2·5	3·8	5·5	6·0	6·5	4·2 cm. in nearly one year	83	9
No. of tips ..	41	82	86	124	..			
4 Length in cm. ..	1·0	1·9	2·9	3·5	4·4	3·4 cm. in earjy one year	28	9
No. of tips ..	12	15	23	40	..			
5 Length in cm. ..	1·5	2·0	3·1	3·2	3·5	2 cm. in nearly one year	27	9
No. of tips ..	21	36	40	48	..			
7 Length in cm. ..	3·0	3·5	5·1	5·9	6·9	3·9 cm. in nearly one year	70	9
No. of tips ..	30	49	83	100	..			

Sections of receptacles examined when dehiscence had begun to wane do show a few new oogonial and antheridial initials arising from the lining of the conceptacles; it may, hence, be presumed that the degeneration of the receptacles is not entirely due to the reproductive potentiality having spent itself but also due to other factors, among which external factors like climatic conditions could be one and effects of parasites another. The receptacles, when nearing maturity, showed on their surface dark dot-like structures; when sectioned and examined they were found to be the fruit bodies of an ascomycetous fungus, of the genus *Mycosphaerella*. A closer examination revealed the mycelium of the fungus traversing the tissues of the plant in the receptacular region (Subrahmanyan, 1957 *a*). It was noticed that during the last few stages of reproduction there was a fall in the number of receptacles which completes the cycle. In fact some of the receptacles which had not dehisced were deteriorating. Very probably, this is due to the injurious effect of the fungus on the host. It may be mentioned here that Sutherland (1915 *a*, 1915 *b*) has recorded several species of fungi occurring in *Pelvetia canaliculata*, and similar records have been made for *Ascophyllum* by Church (1893), Cotton (1908) and David (1943).

A statistical analysis of the cycle of reproduction was made to obtain an idea of the stages of reproduction and their duration. At the beginning of every month 20 typical plants, 10 measuring less than 10 cm. and 10 above 10 cm., were collected and critically examined and the receptacles classified into several categories, stages A, B, C, D, E and F, mentioned already; the number in each category was recorded. (It may be mentioned here that almost all the conceptacles in a receptacle show an identical stage of development.) These stages are represented graphically in Text-Figs. 1 and 2 for plants from Poyll Vaaish and Port Erin respectively. Only the plants measuring over 10 cm. are taken into consideration here. The cycle of reproduction in those of less than 10 cm. was identical, only, those plants contained fewer receptacles. It will be noticed (1) that the curves for the different stages for plants on the two areas are almost similar; (2) that the peak period for the different stages are January, April, June, August-September and October respectively for stages A to E and (3) that stages A, B and C are much drawn out and these are the ones seen from January to July (7 months) while stages D, E and F are compressed into a shorter period, August to October (3 months). The most striking feature, however, is that presented by stage A, that of conceptacle initiation. It will be noticed that in stage A, the peak is attained in about a month, though the period extends up to June, which means that the majority of the plants start on the reproductive phase very early in the season. In order to reach the peak in the initiation in such a short time, the rate of development of the conceptacles must be fairly rapid. Again, only a very careful search reveals the presence of conceptacles so early in the season and none of the earlier papers contain any reference to the period of reproduction in *Pelvetia* beginning as early as this time in the year. Nienburg (1913) in his paper dealing with the development of the conceptacle in the Fucaceae remarks that he could not obtain



TEXT-FIG. 1. *Pelvetia canaliculata*. Graphical representation of the stages of reproduction in plants at Poyll Vaaish. Note secondary peak for stage B, due to plants which started initiation of conceptacles later. Detailed explanation in text.



TEXT-FIG. 2. *Pelvetia canaliculata*. Graphical representation of the stages of reproduction in plants at Port Erin. Detailed explanation in text.

young receptacles of *Pelvetia canaliculata* either from Norway or Plymouth. The facts mentioned above, *viz.*, the difficulty in recognizing such receptacular tips sufficiently early and the rapidity in the development of the conceptacles, are, in the writer's opinion, the reasons why Nienburg probably missed them. The writer himself was unsuccessful several times in his earlier attempts in this direction (*see also* Subrahmanyan, 1957 *a*, p. 13).

Liberation of Reproductive Bodies

Some experiments conducted during the reproductive season and observation on the plants *in situ* showed that there is a more or less regular interval, a fortnightly rhythm, in the liberation of reproductive bodies. These dates were found to coincide with the period of Spring Tides when alone *Pelvetia*, growing at such a high level on the shore, becomes completely submerged; liberation of reproductive bodies otherwise will not be in the interests of the progeny (*refer* Subrahmanyan, 1957 *b*, pp. 374–75 for more details).

General Observations

Juvenile Period

In the foregoing account the plants had been reckoned as they became visible to the unaided eye. But how long will it take for a fertilized egg to grow to a size easily visible to the eye is very difficult to determine. The egg has a diameter varying from 100–137 μ. In the laboratory cultures, the sporelings grow to only 800 μ in about six months (*see also* Subrahmanyan, 1957 *b*, pp. 377 and 384–87). Some experiments were conducted to determine whether the rate of growth for the sporelings is higher or lower on the shore; but, none of them yielded any results.

Rate of Growth‡

Observations of plants in the field and recolonization of cleared areas revealed a few facts. The vegetation is comparatively more luxuriant at lower levels of the same zone particularly evident if the surface is a steep slope. The plants at the bottom attain a larger size than those at the top of the zone and there is a tendency for the plants to be dwarfed at the upper levels; the smallest fruiting plants are invariably noticed here. In the cleared areas, the vegetation showed itself much earlier at the bottom of the area (Area I, for example, which is vertical), than at the top and the denseness of the vegetation also was more emphasized there. This may be attributed to a slight retardation of growth in the upper regions. It was mentioned earlier, that the neap tides very often do not reach and cover the entire *Pelvetia*-zone and the plants are covered completely only by the spring tides; consequently, the plants are left dry for several days and it is not uncommon

‡ The mode of growth and anatomy of the thallus have been described by Subrahmanyan (1956).

to see them in a brittle state. This retarded growth in the upper regions of the zone, therefore, is very likely related to the duration of immersion of the plants by the tides.

Further, an evaluation of the rate of growth recorded with the nature of the surface and position of the areas (*refer* Table II) shows that more plants get settled on a rough surface than a smooth one; more survive on a rough surface in a sheltered place than on smooth and or exposed one; and the rate of growth is greater in a sheltered area at lower levels in the same zone.

Periodicity in Growth

Observations on the larger plants on the shore and plants analysed for reproductive stages during a period of 15 months gave some interesting information. The younger plants show a gradual increase in size (length and bulk) as will be seen from the particulars in Tables II and III. But in the larger plants, during the reproductive period (January to August) when the conceptacles are being formed and the oogonia and antheridia are maturing in them, the vegetative tips of the fronds remain quiescent. Those tips which are on a level with the receptacular tips exhibit this feature very strikingly. On the reproductive activity beginning to wane, these vegetative tips show a remarkable rapid growth in length and overtake the tips which lately formed conceptacles and which were on a level with them (Pl. XIX, Fig. 5).* As the tips of the fronds dichotomise during their growth an increase in their number will indicate whether the plants are actively growing or not. Thees growing tips are much lighter in colour in contrast to their darker tint while quiescent. In the following January when the early signs of conceptacle formation begins on the fronds, vegetative growth, slows down. To illustrate the periodicity in growth, a definite number of plants (ten plants measuring over 10 cm.) were critically examined every month and the number of vegetative tips noted. These data for plants from Port Erin and Poyll Vaaish are shown in Text-Fig. 3, the vegetative tips being represented in percentage. It will be seen from the graph that during Spring and a greater part of Summer the plants show almost no growth; from the beginning of August to the end of December the plants show a rapid rate of growth. Thus, in *Pelvetia*, a definite rhythm in the growth of the plant is noticeable clearly seen in the larger plants, this rhythm becoming evident once the plants start the reproductive cycle.

The growth during this period of vegetative activity in the larger individuals, was also reckoned in a large number of plants; this was facilitated by the presence of the abcissed ends of the fronds which lately formed receptacles remaining on the plant, enabling one to

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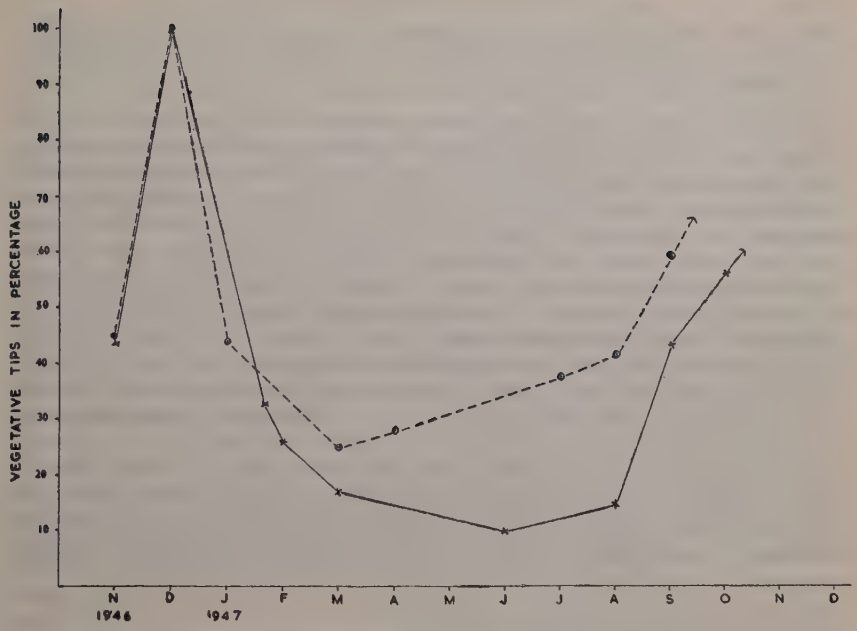
* Often branched receptacles occur (Pl.XIX, Fig. 4), but once conceptacle initiation ends, no further growth in length occurs. The receptacles, however, do grow in thickness and produce considerable quantity of mucilage within.



TEXT-FIG. 3. *Pelvetia canaliculata.* Periodicity in the growth of plants at Port Erin and Poyll Vaaish. Note almost absence of growth from March to August.

measure the increase in length of the vegetative tips (it is to be borne in mind that the quiescent vegetative tips, during the reproductive season, are on a level with the receptacular ones). Making an allowance for the size of the receptacles, which normally does not exceed 1·5 cm., the growth in length of the vegetative tips is within 4 cm. This, again, agrees very nearly with the data obtained from the study of recolonization and that from marked individuals; only, instead of the plant growing throughout the year as the smaller ones do, in the larger individuals, the growth is confined to a shorter period, about five months in the year.

Longevity

It is very difficult to judge the duration of life of the vegetation on the shore. Printz (1926), Nienburg (1930), Rees (1932), David (1943) Knight (1947) and Knight *et* Parke (1950) give some figures for some of the Facaceae other than *Pelvetia.* In the present instance, only some inference is possible on the question of longevity, more so as no reliable information is available on the duration of the juvenile period. The study of the rate of growth, as indicated earlier, gives a rate of about 4 cm. per year for *Pelvetia.* Observations on recolonization also leads to the same conclusion. Taking Area I which the writer considers to be the ideal area compared with the others, it will be seen that the maximum size attained in 17 months by the plants is only 6 cm. To attain

a size of 15–18 cm., the largest of the plants recorded in the normal population cleared on 11th November 1946, it will take four years; in other words, it will take four years for the area to yield a similar population cleared from it.

In this connexion, it may not be out of place to recall here the denudation of the vegetation caused by forces of Nature, conditions obtaining on the shore and effects of parasites. A large number of small individuals are lost in the early stages of recolonization owing to overcrowding and influence of animals like *Littorina* sp. and limpets, and also due to the effect of desiccation; therefore, fewer plants succeed in establishing themselves. Quite a number of adult plants are likely to be lost during storms or by other causes, as witnessed by the disappearance of marked individuals; due to loss of vitality following the production of an enormous crop of receptacles during the reproductive season and finally, effect of fungal parasites which are known to infect *Pelvetia* (Subrahmanyan, 1957 *b*). Taking all these facts into consideration, the writer is inclined to infer the longevity of *Pelvetia* as round about 4 years. Individuals may live longer; at least, there is likely to be, on a given area in the zone, a gradual replacement of the majority of the plants every four years, each such group belonging to a wave of colonization which takes place during successive fruiting seasons.

Reproduction and Vegetative Growth

It was mentioned above, while dealing with the growth of the larger individuals of *Pelvetia*, that a definite rhythm exists in their growth once the plants start reproducing. From January to August, when the reproductive activity of the plants is intense, the vegetative growth is negligible or absent (Text-Fig. 3). From August to December, the plants exhibit a striking acceleration of growth. Thus, it is seen that there is a season of vegetative growth, followed by reproductive activity and these alternate with each other.

Reproduction and Age

In the light of observations on the rate of growth by a study of recolonization of cleared areas and of marked individuals, it may be taken that *Pelvetia* grows in length 3–4 cm. each year. This is a fairly accurate estimate as there are no indications (such as air bladders seen in some of the other Fucaceae) by which one can estimate the age. Even the small plants measuring 4–5 cm., just a year old, are capable of reproducing, but such plants produce only a very few receptacles. These young reproducing plants should not be confused with some of the dwarfed plants of the same size which are often found on the upper regions of the zone and in which one frequently finds all tips transformed into receptacles. The maximum incidence of reproduction, however, is noticed only when the plants are over 8 cm., *i.e.*, 2 years old; and as indicated while discussing the analysis of the normal population, the degree of reproduction measured by the number of receptacles ncreases with the size of (age of) the plants. It may also be added that

the increase in the number of tips transformed into receptacles becomes very markedly higher as the plants increase in size and one very frequently comes across plants in which there are only receptacles and no vegetative tips to continue the growth for, once a tip is transformed into a receptacle, it degenerates at the end of the season. The life of a plant comes to an end when all the tips become transformed into receptacles during a reproductive season unless some proliferations arise or those that are already present continue the growth. One not infrequently comes across such plants but, it is doubtful whether they continue for any length of time. The proliferations appear to be vigorous only when they are on young plants or if they arise as a result of an injury to a young plant, which appears to be the case with the one represented in Pl. XIX, Fig. 8, where the plant simulates a normal one. Decapitation of young plants confirmed this feature in the course of experiments on the shore.

Thus, it is seen that a normal plant has a span of life of about four–five years which consists of an active vegetative period of over two years during which time it increases in size and bulk, then a period when vegetative growth and reproductive phase alternate with each other and as the plant becomes aged vegetative growth slackens and the plant spends itself by producing receptacles and finally disappears from the scene.

Harvesting

After a study of *Pelvetia canaliculata* during the course of two years, the writer is inclined to conclude that removal of the vegetation comprising plants measuring less than 14 cm. in length up to three years old plants—will seriously affect the productivity in this zone in the long run. It is advisable to remove only the larger ones which are over 14 cm. in length; for, in the fourth year the reproductive potentiality is on the decline as well as vegetative growth. The content of economically useful products, such as alginic acid, in plants of different categories will have to be determined before deciding the group of plants to be harvested.

Variations

It will be interesting to record here some variations met with. Plants obtained from Millport and Aberdeen were identical to those growing in the Isle of Man though somewhat darker in tint. The plants growing at Langness in the Isle of Man, where a lot of mud is found deposited, showed a resemblance to those described by Baker (1912) as *Pelvetia canaliculata* var. *libera* from salt marshes, in having a bushy appearance with the frondage a little thinner and interlaced. Baker found them free floating unlike in the present locality where they were attached. Skrine (1929) also describes a salt marsh form of *Pelvetia*.

A few plants met with during the reproductive season showed some stray conceptacles occurring far below the usual receptacular ends, separated by a gap of, at times, 2 cm. of vegetative tissue (Pl. XIX,

Fig. 3). This is probably due to the fact that after a few conceptacles had been initiated, vegetative growth intervened before initiation of more conceptacles. Lami (1938, pp. 180–81) cites such an instance and states that after differentiation of an " intercalary conceptacle ", the extremity grows and produces new receptacles and in one instance found three such successively. He is inclined to attribute it to an ecological origin. He names the plant *P. canaliculata* forma *interposita* Lami; this form is not rare in the region of Minho and Hamel, according to Lami, has also observed it in Galice in 1927.

Again, during the course of the present investigation, two or three instances of receptacles with a tapering extremity were observed (Pl. XIX, Fig. 6). Sections showed only stray conceptacles in this tapered region. The conceptacles were smaller than the normal ones and occurred towards the base. This type of receptacles were present along with the usual ones on the same plant. Here also, it would appear that vegetative growth had taken place after initiation of some conceptacles by the apical cell. The similarity of these to those found in *P. canaliculata* var. *acutilobata* described by Lami (1938, pp. 180–81, Fig. 2) is worth mentioning (*see also* Subrahmanyan, 1957*a*, p. 16).

Summary

Observations on the ecology of *Pelvetia canaliculata* Dcne. *et* Thur. on the Isle of Man are described in some detail. The account deals with the repopulation of five areas in the *Pelvetia*-zone situated at two different localities. The normal population cleared from the areas was classified and analysed qualitatively and quantitatively. The succession of forms on the cleared areas was followed and characteristic form on them recorded. One area was re-cleared after a length of time and analysed. Individual plants also were marked and observed at intervals.

The rate of growth for *Pelvetia* was found to be 4 cm. length a year and the longevity about 4 years. The rate varied with the height at which the alga grew in relation to tide levels, the relation being inverse. The denseness of the population appeared to depend upon tide levels, nature of surface and degree of shelter the area had from sun and waves.

The alga has a period of vegetative growth lasting about 2 years and with the onset of reproduction in the third year or so, a rhythm sets in, a period of vegetative growth alternating with a period of reproduction. Finally, vegetative growth declines, all the apices end in receptacles and with no more vegetative " leaders " left to continue growth, the plant dies out.

First signs of reproduction are noticed in January; the period lasts till September, the peak as witnessed by the discharge of reproductive bodies begins in August and lasts till middle of September. Six stages were distinguished during the reproductive cycle, the period of initiation of conceptacles being short and rapid. There appear to be

a relationship between Spring tides and liberation of reproductive bodies. The relation between age and reproduction is indicated.

Acknowledgement

The writer wishes to express his gratitude to the Government of India for the award of an Overseas Scholarship which made the research of which the foregoing is a record possible; to Prof. John McLean Thompson, M.A., D.Sc., F.L.S., F.R.S.E., Halbrook Gaskell Professor of Botany (now retired) in the University of Liverpool, who facilitated the research in every way; to Dr. Margery Knight, D.Sc., F.L.S., for advice in the prosecution of the research; and to Mr. J. R. Bruce, M.Sc., of the Marine Biological Station, Port Erin, for valuable help during his visits to the Isle of Man.

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EXPLANATION OF PLATE XIX

Pelvetia canaliculata. Plants of varying ages and sizes in different stages of reproduction. Stages shown by letters A, B, C, D, E and F. Detailed explanation in text. Note however in: Fig. 3: stray conceptacles "*g*" far below the receptacular portion; Fig. 4: "branched receptacles"; Fig. 5: vegetative tips "*v*" grown past the receptacular ones "*r*" which are degenerating after period of reproduction; Fig. 6 : receptacles with tapering apices; and Fig. 8: bunch of proliferations from the apex of a frond, some with receptacles "*h*".

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R. Subrahmanyan FIGS. 1-8

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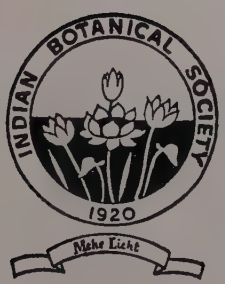
PROCEEDINGS
OF THE
ANNUAL MEETING, 1960



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THE INDIAN BOTANICAL SOCIETY
MINUTES OF THE THIRTY-NINTH ANNUAL MEETING OF THE GENERAL BODY

The Thirty-Ninth Annual General Body Meeting of the Indian Botanical Society was held on 3rd January 1960, at 2–15 P.M., in the Convocation Hall, University of Bombay, Bombay.

The following members were present:—

Prof. A. Abraham, Prof. J. C. Sen Gupta, Dr. B. M. Johri, Dr. S. K. Pande, Dr. Agharkar, Prof. P. Parija, Dr. P. N. Mehra, Prof. P. Maheshwari, Prof. J. Venkateswarlu, Prof. T. S. Sadasivan, Prof. R. Misra, Dr. E. K. Janaki Ammal, Dr. A. C. Joshi, Mr. R. S. Chopra, Dr. G. S. Puri, Dr. S. C. Pandeya, Mr. G. P. Agarwal, Mr. S. Saksena, Mr. C. S. Venkatesh, Dr. K. Subramanyam, Rev. Fr. H. Santapau, Dr. K. B. Deshpande, Dr. Bahdur Singh, Dr. B. C. Kundu, Dr. C. V. Subramanian, Dr. A. T. Zachariah, Dr. T. V. Desikachary, Mr. D. D. Awasti, Mr. G. P. Panigrahi, Dr. Narayana, Dr. A. K. Mitra, Prof. R. M. Desai, Mr. P. D. Bhati, Mr. P. V. Bole, Mr. R. K. Gupta, Dr. K. S. Thind, Prof. R. P. Roy, Mr. S. K. Jain, Mr. R. R. Das, Mr. I. P. Bahri, Mr. S. K. Wagh, Mr. S. T. Tilak, Mr. D. N. Sen, Mr. S. K. Singh Raikwar, Dr. S. Chitaley, Dr. K. A. Chowdhary, Mr. H. P. Mehta, Mr. M. Sukla, Mr. S. S. Kelkar, Mr. N. D. Kamat, besides several visitors including Dr. J. Eipe.

1. The members of the Indian Botanical Society place on record their deep sense of sorrow at the sad demise of Prof. G. P. Majumdar, Life-Member of the Society, and express their sincerest condolences to the members of the bereaved family.

This meeting of the Indian Botanical Society records its sense of grief at the passing away of Mrs. D. D. Baria, one of its Life-Members, and wishes to express its heart-felt sympathy to the members of the bereaved family.

The Indian Botanical Society has learnt with deep regret the sad demise of Rev. A. Rapinat and expresses their sincerest condolences to the members of the bereaved family.

Resolved that the bereaved families be informed of the resolutions.

2. The minutes of the 38th General Body Meeting held at Delhi on 21st January 1959, at 2–45 P.M., were read and confirmed.

3. The Annual Report of the Society for the year 1959 as proposed by Prof. R. Misra and seconded by Rev. Fr. H. Santapau in the Executive Council Meeting was read and confirmed.

4. The Audited Statement of Accounts for the period from 1–4–1958 to 31–3–1959 already circulated as Proceedings of the Society, 1959 to the members in the Journal (Vol. 38, No. 3) of the Society was considered and approved.

The Budget Estimates for the year 1960–61 were presented by Prof. T. S. Sadasivan, Hon. Treasurer, Indian Botanical Society. The same were considered by the House and passed.

5. (*a*) Prof. P. Parija, Prof. L. F. Randolph, Mr. F. C. Bawden and Prof. Dr. K. Mothes were elected Honorary Members of the Society for their eminent contributions to Botany.

(*b*) The following applicants were admitted to the Society as new members subject to their payment of admission fee and annual subscription:—

1. Dr. B. S. Sivarao, Waltair.
2. Dr. A. P. Misra, Sabour.
3. Mr. S. C. Mathew, Jaipur.
4. Mr. R. S. Dwivedi, Varanasi-5.
5. Mr. A. C. Gupta, Dehra Dun.
6. Dr. B. S. Mehrotra, Allahabad.
7. Miss T. Saraswati, Bangalore.
8. Mr. R. S. Rana, Delhi.
9. Prof. V. S. Rao, Bombay.
10. Mr. John Parkin, England.

(*c*) The following donors to the Society were thanked for the annual grants-in-aid generously given by them for the year 1959–60:—

		Rs.
1.	Madras University	250
2.	Banaras Hindu University	250
3.	Utkal University	250
4.	University of Kerala	250
5.	Andhra University	100
6.	National Institute of Sciences of India	2,000

6. The President for the year 1959, Dr. E. K. Janaki Ammal, delivered her address to the Society and at the end of the address, a vote of thanks for the same was passed by the General Body.

7. The results of the election for the year 1960 given below were announced by the President:—

President: Dr. I. Banerji, Calcutta.

Vice-Presidents: 1. Dr. E. K. Janaki Ammal, Jammu; 2. Prof. R. Misra, Varanasi-5.

Hon. Secretary: Prof. J. Venkateswarlu, Waltair.

Hon. Librarian: Prof. R. Misra, Varanasi-5.

Hon. Treasurer: Prof. T. S. Sadasivan, Madras.

Councillors: (1) Dr. I. Banerji, Calcutta; (2) Prof. S. N. Das Gupta, Lucknow; (3) Dr. A. C. Joshi, Chandigarh; (4) Dr. P. Parija, Cuttack; (5) Prof. V. Puri, Meerut; (6) Prof. T. S. Mahabale, Poona; (7) Mr. M. B. Raizada, Dehra Dun; (8) Prof. S. Ranjan, Allahabad; (9) Prof. R. P. Roy, Patna; (10) Rev. Fr. H. Santapau, Bombay.

Business Manager: Prof. T. S. Sadasivan, Madras.

Members of the Editorial Board: (1) Dr. A. C. Joshi, Chandigarh (1957–60); (2) Rev. Fr. H. Santapau, Bombay (1958–61); (3) Prof. P. Maheshwari, Delhi (1959–62); (4) Dr. B. P. Pal, Delhi (1960–63).

8. The retiring Office-bearers were appropriately thanked on a motion by the Hon. Secretary, Prof. J. Venkateswarlu. The President thereupon thanked the Hon. Secretary in appreciation of his services to the Society during 1956–60.

The authorities of Bombay University, Indian Science Congress Association and particularly Prof. R. D. Adatia of Bhavan's College and Rev. Fr. H. Santapau of St. Xaviers College of Bombay were thanked for all the help rendered by them in connection with the meetings and functions of the Indian Botanical Society.

A group photograph of the members of the Indian Botanical Society present was taken on 5th January 1960.

ANNUAL REPORT, 1959

THE Executive Council of the Indian Botanical Soicety have pleasure in submitting the following report for the year 1959–60:—

1. The 38th Annual General Body Meeting of the Society was held on 21st January 1959, at 5–30 P.M., in New Botany Lecture Theatre (F), Botany Department, University Buildings, Delhi-8, with Prof. R. Misra in Chair.

The Minutes of the meeting are being published as proceedings of the Indian Botanical Society in one of the coming issues of Volume 38 of the *Journal of the Indian Botanical Society* for the year 1959–60.

Owing to sharp rise in the cost of printing and paper in recent days it was thought desirable to adopt certain measures of economy in the running of the Society and to increase revenue of the Society. Upon the recommendations of Executive Council the rules of the Society [Rule No. 8, 16, 24, 29 (*a*), 31 and 33] were revised and they are now read as printed and circulated to the members of the Society along with one of the early issues of Volume 38 of *Journal of the Indian Botanical Society*.

According to the result of the Election, the following authorites were constituted for the year 1959:—

President: Dr. E. K. Janaki Ammal.

Vice-Presidents: Prof. R. Misra; Prof. P. Maheshwari.

Hon. Secretary: Prof. J. Venkateswarlu.

Treasurer: Prof. T. S. Sadasivan.

Editor-in-Chief: Prof. T. S. Sadasivan.

Councillors: Dr. I. Banerji; Dr. A. C. Joshi; Prof. S. N. Das Gupta; Prof. T. S. Mahabale; Dr. P. Parija; Mr. M. B. Raizada; Prof. S. Ranjan; Prof. R. P. Roy; Rev. Fr. H. Santapau.

Members of the Editorial Board: Dr. B. P. Pal; Dr. A. C. Joshi; Rev. Fr. H. Santapau; Prof. P. Maheshwari.

2. A meeting of the Executive Council was held in Liabrary Room, Department of Botany, University Buildings, Delhi-8, at 11 A.M., on Wednesday, the 21st January 1959. Matters relating to the publication of *History of Botanical Research in India, Burma and Ceylon* and *Vegetational Types of India* were discussed and it was *resolved* that the publication of these articles be expedited and at least four more special publications may be brought out during the year 1959–60.

3. An increase in the clerical allowance from Rs. 80 to Rs. 100 p.m. at the Office of the Treasurer and Business Manager and Editor-in-Chief at Madras was considered and passed.

4. A meeting of the Editorial Board of the *Journal of the Indian Botanical Society* was held in the Library Room, Botany Department, University Buildings, Delhi, on 21st January 1959, at 1 P.M. Prof. T. S. Sadasivan was elected as Editor-in-Chief and Business Manager for the year 1959.

5. A group photograph of the members of the Indian Botanical Society, who attended the Annual Meeting, was taken on 21–1–1959 at Botany Department, Delhi University, Delhi-8.

6. At Dehra Dun Branch of the Society on 5th January, 1959, Dr. B. L. Burtt, Botanist at Royal Botanical Gardens, Edinburgh, gave an interesting and informative talk on "Modern Taxonomy in relation

to General Botany". On 3rd March 1959, Prof. E. J. H. Corner gave a general talk on "The value of the study of 'Systematic Botany'". Both meetings were attended by a large number of special invitees besides the members of the Branch. The meetings were presided over by Shri S. S. Ghosh, Officer-in-Charge, Wood Anatomy Branch.

7. In accordance with conditions of the generous endowment made by Prof. T. S. Sadasivan, the Executive Council has decided to award Birbal Sahni Medal for the year 1959 to Prof. P. Parija for his outstanding contribution in Plant Physiology and for his services to the cause of Indian Botany.

8. The following were published during the year:—

Vol. 38 [Nos. 1, 2, 3 (already mailed), and 4 (in Press)].

9. Membership:—

Honorary Members	8
Life-Members	91
Ordinary Members	391
TOTAL	490
Admissions during 1959	45
Resignations	5
Not of good standing	11

10. Subscribers:—

Indian Institutions	122
Foreign	126
Free exchange	48